REMARKS

Claims 8, 13-16, 31, 49 and 50 are pending in this application. Claims 14-16 are withdrawn from consideration.

As requested by the Examiner, a clearer version of claim 13 has been presented.

The Applicants have amended claims 8, 49 and 50. Claims 14-16 remain withdrawn. The Applicants respectfully note that the withdrawn claims have also been amended.

Additionally, the Applicants have added new claims 51-53 to the present application. The Applicants respectfully submit that the new claims do not add subject matter to the present application. Support for new claim 51 can be found in claim 8 and in the specification, particularly, in Example 23.

Upon entry of the present amendment, claims 8, 13, 31 and 49-53 are pending in this application and claims 14-16 are withdrawn.

I. Rejections under 35 U.S.C. 102

The Office has rejected claim 8 under 35 U.S.C. 102(b), as being anticipated by Howard (EP '435). Specifically, the Office has stated that at least one species within claim 8 is anticipated by Howard.

Based on the amendment of claim 8 as set forth in the present amendment, including the cancellation of 6-[3-(4-1,2-benzisothiazol-3-yl-piperazin-1-yl)-propyl]-4-methyl-3,4-dihydro-1H-quinolin-2-one, the Applicants respectfully submit that the rejection under 35 U.S.C. 102(b) has been rendered moot.

II. Rejections under 35 U.S.C. 103

The Office has rejected claims 8, 13, 31 and 49-50 under 35 U.S.C. 103(a), as being unpatentable over Howard (EP '435).

Based on the argument and comparative data presented below, the Applicants respectfully submit that the subject matter of the amended claims is patentable over Howard.

Atypical antipsychotics show improved efficacy with fewer side effects compared to first generation typical antipsychotics. While both typical and atypical antipsychotics are potent antagonists of dopamine D_2 receptors, most atypical antipsychotics have affinity for serotonin (5-HT) receptors (Meltzer, 1999; Werkman et al, 2006). Affinity for the 5-HT_{2A} subtype of serotonin receptors is thought to contribute to the lower incidence of extrapyramidal side effects as well as additional efficacy against both positive and negative symptoms associated with schizophrenia (Scanton and Sanger, 2000). In fact, most atypical antipsychotics have higher affinity for 5-HT_{2A} receptors relative to D_2 receptors as measured in receptor binding assays; a feature thought to be important for the atypical antipsychotic profile (Meltzer, et al, 1989; Meltzer, 1999).

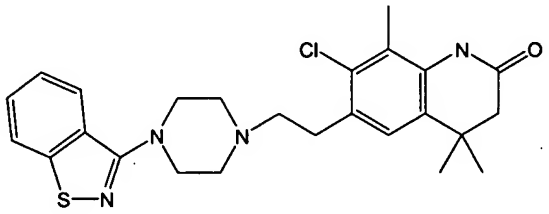
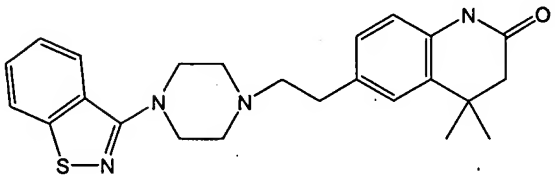
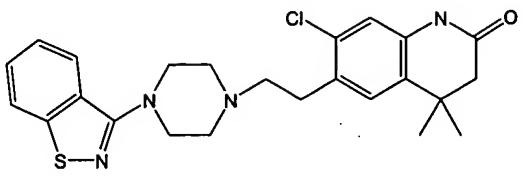
The compound of claim 51 and free base of claim 8, 6-[2-(4-benzo[d]isothiazol-3-yl-piperazin-1-yl)-ethyl]-7-chloro-4,4,8-trimethyl-3,4-dihydro-1H-quinolin-2-one (Compound 1), differentiates from the closely related compounds of Howard (Compounds of examples 45, 51 and 49, respectively) by having a 3.3 to 4.8-fold higher affinity at serotonin 5-HT_{2A} receptors and a 14 to 19-fold higher D_2/5-HT_{2A} ratio (Table 1).

Likewise, the compound of claim 49 and free base of claim 50, 6-[2-(4-benzo[d]isothiazol-3-yl-piperazin-1-yl)-ethyl]-8-chloro-4,4-dimethyl-3,4-dihydro-1H-

quinolin-2-one (Compound 2) differentiates from the closely related compounds of Howard (Compounds of examples 45, 51 and 49, respectively) by having a 4.7 to 7.6-fold higher affinity at serotonin 5-HT_{2A} receptors and a 47 to 65-fold higher D_2/5-HT_{2A} ratio (Table 2).

These profiles demonstrate that the compounds of the claimed invention have superior efficacy with the expectation of a reduced side-effect profile as compared to the compounds of Howard.

Table 1. Inhibitory affinity constants (Ki) for Compound 1 and related compounds on dopamine D_2 and serotonin 5-HT_{2A} receptors as measured in radioligand receptor binding assays

Compound	Structure	D_2 Ki (nM)	5-HT_{2A} Ki (nM)	D_2/5-HT_{2A} ratio
1		6	0.1	60
e.g. 45 in Howard		1.5	0.48	3.1
e.g. 51 in Howard		2.3	0.53	4.3
e.g. 49 in Howard		1.3	0.33	3.9

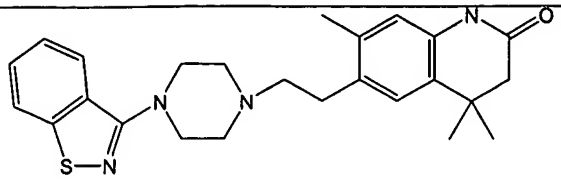
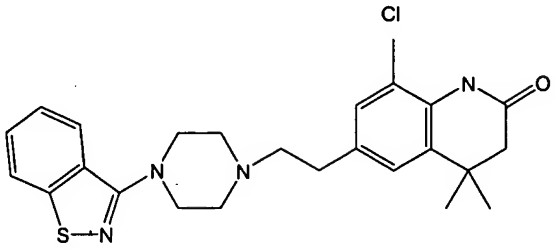
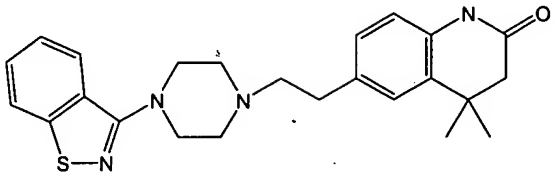
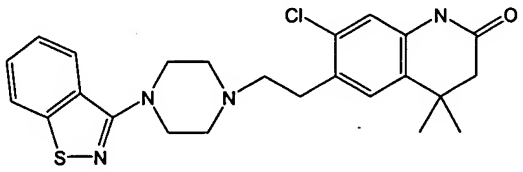
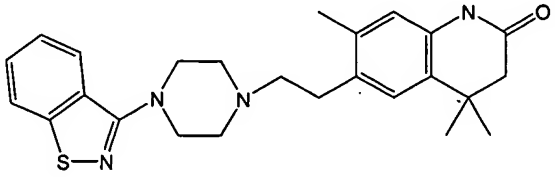
				

Table 2. Inhibitory affinity constants (Ki) for Compound 2 and related compounds on dopamine D_2 and serotonin 5-HT_{2A} receptors as measured in radioligand receptor binding assays

Compound		D_2 Ki (nM)	5-HT_{2A} Ki (nM)	D_2/5-HT_{2A} ratio
2		14	0.07	200
e.g. 45 in Howard		1.5	0.48	3.1
e.g. 51 in Howard		2.3	0.53	4.3
e.g. 49 in Howard		1.3	0.33	3.9

The following references referred to in the argument provided above are attached for your convenience:

Meltzer H.Y. The role of serotonin in antipsychotic drug action. Neuropsychopharm 1999; 21(2S):106S-15S.

Scanton, B. and Sanger, D.J. Pharmacological and molecular targets in the search for novel antipsychotics. Behavioural Pharmacology. 2000; 11:243-256.

Meltzer, H.Y., Matsubara, S., Lee, J-C. Classification of typical and atypical antipsychotic drugs on the basis of dopamine D_1, D_2, and serotonin$_2$ pK_i values. J. Pharmacol. Exp. Ther. 251:238-46, 1989.

Werkman T.R., Glennon, J.C., Wadman, W.J. and McCreary, A.C., Dopamine receptor pharmacology: Interactions with serotonin receptors and significance for the aetiology and treatment of schizophrenia. CNS & Neurological Disorders – Drug targets. 2006; 5: 2-23.

Based on the foregoing, the Applicants respectfully submit that the claimed subject matter is patentable over Howard and request that the rejection under 35 U.S.C. 103(a) be withdrawn.

III. Provisional Rejection under under Obviousness-type double patenting

The Office has stated that claims 8, 13, 31 and 49-50 are provisionally rejected on the group of nonstatutory obviousness-type double patenting as being unpatentable over claims of copending Application Serial No. 10/672,949.

The Applicants hereby state that the instant application and copending were commonly owned at the time the instant invention was made.

The Applicants hereby acknowledge the provisional rejection under the judicially created doctrine of obviousness-type double patenting. No terminal disclaimer will be filed at this time.

IV. Conclusion

Upon entry of the present amendments, the Applicants submit that this application is now in condition for allowance, which allowance is respectfully solicited.

If the Examiner believes that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at 734-622-2658.

Respectfully submitted,

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Classification of Typical and Atypical Antipsychotic Drugs on the Basis of Dopamine D-1, D-2 and Serotonin$_2$ pK$_i$ Values[1]

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ABSTRACT

The pK$_i$ values of 13 reference typical and 7 reference atypical antipsychotic drugs (APDs) for rat striatal dopamine D-1 and D-2 receptor binding sites and cortical serotonin (5-HT$_2$) receptor binding sites were determined. The atypical antipsychotics had significantly lower pK$_i$ values for the D-2 but not 5-HT$_2$ binding sites. There was a trend for a lower pK$_i$ value for the D-1 binding site for the atypical APD. The 5-HT$_2$ and D-1 pK$_i$ values were correlated for the typical APD whereas the 5-HT$_2$ and D-2 pK$_i$ values were correlated for the atypical APD. A stepwise discriminant function analysis to determine the independent contribution of each pK$_i$ value for a given binding site to the classification as a typical or atypical APD entered the D-2 pK$_i$ value first, followed by the 5-HT$_2$ pK$_i$ value. The D-1 pK$_i$ value was not entered. A discriminant function analysis correctly classified 19 of 20 of these compounds plus 14 of 17 additional test compounds as typical or atypical APD for an overall correct classification rate of 89.2%. The major contributors to the discriminant function were the D-2 and 5-HT$_2$ pK$_i$ values. A cluster analysis based only on the 5-HT$_2$/D$_2$ ratio grouped 15 of 17 atypical + one typical APD in one cluster and 19 of 20 typical + two atypical APDs in a second cluster, for an overall correct classification rate of 91.9%. When the stepwise discriminant function was repeated for all 37 compounds, only the D-2 and 5-HT$_2$ pK$_i$ values were entered into the discriminant function. These data suggest determination of D-2 and 5-HT$_2$ pK$_i$ values may be useful for rapid screening of candidate atypical APDs with only minimal false positives. The implications of these findings for the mechanism of action of atypical APDs is discussed.

Clozapine has been shown to be a more effective APD than chlorpromazine in two recent multicenter clinical trials (Claghorn *et al.*, 1987; Kane *et al.*, 1988). Clozapine also produces less acute EPS than typical APDs (Matz *et al.*, 1974), and does not elevate serum PRL levels in humans (Meltzer *et al.*, 1979). There have been no reliable reports that clozapine produces or exacerbates TD, although it can block the symptoms of TD (Meltzer and Luchins, 1984). It has been suggested that a common biological effect of clozapine on neurotransmitter system(s) may be relevant to all these clinical advantages (Meltzer, 1989). Early theories concerning the mechanism of action of clozapine have been reviewed elsewhere (Meltzer, 1989).

The antagonist action of typical neuroleptic drugs, as well as clozapine, for the DA D-2 receptor (negatively coupled to adenylate cyclase) in the mesolimbic and mesocortical DA systems has been considered to be the major basis of their antipsychotic action (Meltzer and Stahl, 1976; Seeman *et al.*, 1976). There is no evidence that any of the typical neuroleptics are more effective as antipsychotic agents than any other (Baldessarini, 1985). However, the affinity of clozapine for the D-2 receptor *in vitro* correlates with average clinical dose on the same regression line as other neuroleptic drugs (Seeman, 1980), whereas *in vivo* it is, if anything, weaker as a striatal D-2 antagonist than would be predicted on the basis of this regression (Fardé *et al.*, 1988, 1989). It is therefore unlikely that the D-2 antagonistic effect of clozapine can explain its superior efficacy. This will be addressed further under "Discussion."

Recent evidence suggesting an important influence of agonists and antagonists for DA D-1 receptors (positively coupled to adenylate cyclase) on a variety of behaviors that are influenced by D-2 receptor stimulation has been reviewed by Clark and White (1988). This, plus several biochemical and behavioral studies showing an effect of clozapine on various D-1 mechanisms (Andersen *et al.*, 1986; Andersen and Braestrup, 1986; Rupniak *et al.*, 1985; Chipkin and Latranyi, 1987; Altar *et al.*, 1988; Gudelsky and Meltzer, 1989), has led to the hypothesis that the effect of atypical APD on D-1 receptors, either absolutely or relative to their effect on D-2 receptors, could be of critical importance for the clinical advantages of

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ABBREVIATIONS: APD, antipsychotic drugs; EPS, extrapyramidal symptoms; PRL, prolactin; TD, tardive dyskinesia; DA, dopamine; D-1 and D-2, DA receptor subtypes; 5-HT, 5-hydroxytryptamine (serotonin).

APDs both for EPS (Andersen and Braestrup, 1986; Chipkin and Latranyi, 1987) or their antipsychotic action (Altar *et al.*, 1988).

The effect of atypical APDs on 5-HT receptors has also been suggested to be relevant to their unique properties. There is considerable evidence that clozapine is a potent 5-HT antagonist *in vivo* (Sulpizio *et al.*, 1978; Fjalland, 1979; Lai *et al.*, 1980; Fink *et al.*, 1984; Nash *et al.*, 1988). Both Sulpizio *et al.* (1978) and Lai *et al.* (1980) suggested that the antiserotonergic properties of clozapine, in relation to its antidopaminergic properties, might account for its lack of EPS as there is considerable evidence that 5-HT antagonists diminish the ability of neuroleptic drugs to produce catalepsy (Costall *et al.*, 1975; Costall and Naylor, 1978; Waldmeier and Delini-Stula, 1979). Similar suggestions have been made by Altar *et al.* (1986b) and Rasmussen and Aghajanian (1988).

Thus, whether the D-1 or 5-HT_2 antagonist properties of atypical APDs, both or neither, in addition to their D-2 antagonism, are relevant to the atypical properties of clozapine-like drugs is controversial. Part of the difficulty in evaluating these hypotheses is that the studies cited above examined relatively few typical or atypical APD. Secondly, as noted above, there is ambiguity about classification of some APDs as typical or atypical. A third problem is that many APDs, e.g., SCH 23390, trifluoperazine, fluphenazine, perphenazine, prochlorperazine, *cis*-flupentixol, (+)-butaclamol, RMI 81582, etc., have strong affinities for both D-1 and 5-HT_2 receptors (see tables 1 and 6).

In this study we measured the affinities of a large group of typical and atypical APDs for rat striatal D-1 and D-2 and cortical 5-HT_2 receptor binding sites to determine the importance of D-1, D-2 and 5-HT_2 absolute or relative affinities in distinguishing these two classes of APDs. Drugs were classified as atypical if: 1) clinical trials indicated antipsychotic activity with minimal extrapyramidal side effects; 2) clinical experience suggests no causation of TD or small elevation of serum PRL levels; or 3) if preclinical studies demonstrated no or weak cataleptic potential.

We first examined a group of 13 typical and 7 atypical APDs (Table 1) for which there is quite good clinical data, to classify them as typical or atypical. All compound which produce large increases in serum PRL levels or high EPS, or both, were classified as typical. These are designated as the *reference* compounds in the rest of this report. They were used to develop a discriminant function which was then used to classify 17 other drugs (table 6) which are less certainly classified as atypical or typical APD. These 17 compounds comprise the *test* group. The test compounds were *a priori* classified as typical or atypical on the basis of the clinical criteria cited above, if available, or on the basis of producing weak or no catalepsy in rodents. The classification of the reference and test compounds on the basis of the clinical and preclinical evidence was then compared with that provided by the discriminant function. It should be noted that all compounds were classified as typical or atypical before determination of their D-1, D-2 or 5-HT_2 affinities. To confirm our results, a cluster analysis of the compounds based only on the ratio of the pK_i values for the 5-HT_2 and D-2 receptor binding sites was carried out.

Materials and Methods

Materials. Male, Sprague-Dawley rats weighing 200 to 250 g (Zivic Miller Laboratories, Hilbon, PA) were used. The rats were sacrificed by decapitation. Dissected striata and frontal cortex were frozen on dry ice and stored at −80°C until used.

[^{3}H]SCH 23390 (66–70 mCi/mmol), [^{3}H]spiperone (26.8 mCi/mmol) and [^{3}H]ketanserin (61–61.9 mCi/mmol) were purchased from DuPont New England Nuclear (Boston, MA). Chlorpromazine was purchased from Sigma Chemical Co., St. Louis, MO). The other drugs were generously provided by the manufacturers: (+)-butaclamol (Ayerst Laboratories, New York, NY) molindone (Endo Laboratories, Garden City, NY); pipamperone, spiperone, ritanserin, moperone, benperidol, ketanserin and setoperone (Janssen Pharmaceutica, Beerse, Belgium); amoxapine and loxapine (Lederle Laboratories, Pearl River, NY); melperone, FG 5803 and amperozide (Pharmacia, Malmö, Sweden); *cis*-flupentixol (Lundbeck, Copenhagen, Denmark); haloperidol and pimozide (McNeil Laboratories, Inc., Fort Washington, PA) tiospirone (Mead Johnson and Co., Evansville, IN); RMI-81582 (Merrell Dow Pharmaceuticals, Cincinnati, OH); thiothixene (Pfizer Inc., New York, NY): clozapine, fluperlapine, perlapine, clothiapine, mesoridazine and thioridazine (Sandoz Pharmaceuticals, Hanover, NJ); chlorpromazine, prochlorperazine and trifluoperazine (Smith Kline and French Laboratories, Philadelphia, PA); fluphenazine (E. R. Squibb & Sons, Princeton, NJ): SCH 23390 and perphenazine (Schering-Plough, Bloomfield, NJ); tenilapine and rilapine (Knoll Pharmaceutical Co., Orange, NJ); CGS 10746 (Ciba-Geigy, Summit, NJ), HP 370 (Hoechst-Roussel Pharmaceutical Co., Somerville, NJ); methiothepine (Hoffmann-La Roche, Inc., Nutley, NJ); and zotepine (Fujisana Pharmaceutical Co., Tokyo, Japan).

Methods. Homogenates of striatal or cerebral cortical tissue from rats were prepared in 100 volumes (W/V) or 50 volumes, respectively, of 50 mM Tris-HCl (pH 7.7 at 25°C) containing 5 mM EDTA with a Polytron setting of 6 (20 sec). The homogenates were centrifuged twice (48,000 $g \times 10$ min at 4°C) with intermediate resuspension in 50 mM Tris-HCl buffer (pH 7.4 and pH 7.7 for striatal and cortical tissues, respectively) at 25°C and centrifuged again. The final pellets were resuspended in these buffers before use in the binding assay.

The assays were carried out according to the methods of Billard *et al.* (1984) for DA D-1 (Mikuni *et al.*, 1984), for DA D-2 (Stockmeier and Kellar, 1986) and for 5-HT_2 receptor binding with modifications as described below. All determinations were done at least in duplicate.

Aliquots of the membrane preparation were incubated with [^{3}H]SCH 23390 (for DA D-1 receptor binding) or [^{3}H]spiperone (for D-2 binding) for 15 min at 37°C in 50 mM Tris-HCl (pH 7.4 at 25°C) containing (in millimolar): NaCl, 120; KCl, 5; $CaCl_2$, 2; and $MgCl_2$, 1. The assay mixture for D-2 binding also contained 0.1% ascorbate and 50 nM ketanserin (to block 5-HT_2 binding sites). Aliquots of cortical membrane preparations were incubated with [^{3}H]ketanserin (for 5-HT_2 receptor binding) for 15 min at 37°C in 50 mM Tris-HCl (pH 7.7). The final tissue concentrations were 1 mg of original wet weight tissue per 2 ml for [^{3}H]SCH 23390 binding, 2 mg/3 ml for [^{3}H]spiperone binding and 5 mg/2 ml for [^{3}H]ketanserin binding. In preliminary association, experiments, equilibirum was established by 10 min for the D-1 and D-2 assays (data not presented). Under similar assay conditions, equilibrium was demonstrated with [^{3}H]ketanserin by 5 min (Leysen *et al.*, 1982).

The incubation was terminated by rapid filtration over Whatman GF/B (for D-1 and D-2) or GF/C (for 5-HT_2) filters. The filters were rinsed 3 times with 5 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°C).

Nonspecific binding was determined in the presence of 1 μM *cis*-flupentixol (for D-1 binding), 1 μM (+)-butaclamol (for D-2 binding) or 2 μM methysergide (for 5-HT_2 binding).

IC_{50} values for the displacement of [^{3}H] SCH 23390 (0.5 nM), [^{3}H] spiperone (0.2 nM) or 0.5 nM [^{3}H]ketanserin were determined by log-probit analysis of data from inhibition experiments in which nine different concentrations of drugs spanning three orders of magnitude were used. Nonspecific binding at these concentrations of ^{3}H-ligands were about 10% (for D-1), 18% (for D-2) and 11% (for 5-HT_2) of total binding (about 5,000 dpm for D-1, 2,900 dpm for D-2 and 10,800 dpm for 5-HT_2).

K_i values were calculated according to the equation: $K_i = IC_{50}/(1+L/K_D)$ with L the concentration and K_D the apparent dissociation constant of the ^{3}H-ligand obtained from Scatchard analysis of saturation experiment data. Each K_i value was determined at least in duplicate. The mean coefficient of variation for duplicate determinations of the K_i value for the three types of binding sites was 14.5 ± 4.1% (S.D.).

Data Analysis. In order to normalize the data for statistical analysis, the (−)-log of the K_i values (pK_i) was used in all calculations. The mean pK_i values of the 13 reference typical compounds for the D-1, D-2 and 5-HT_2 binding sites and the ratios of the pK_i values for the 5-HT_2/D-2, 5-HT_2/D-1 and D-1/D-2 binding sites were compared with those of the seven atypical drugs by analysis of variance. The correlations between the pK_i values of the three binding sites were determined for all compounds and the two groups (Spearman rho). We next determined the contribution of each pK_i to the discrimination of the two classes of drugs through a stepwise discriminant function using the SAS program, STEPDISC. (Discriminant analysis is useful in deciding which combination of variables is most important in predicting group membership. Discriminant function generates a linear combination of those variables such as the pK_i values for D-1, D-2 and 5-HT_2 binding sites, which potentially can distinguish between two populations when appropriate weightings are applied.) In one type of discriminant function analysis, stepwise selection enters the variable which contributes most to the discriminatory power of the model as measured by Wilk's *lambda*, the likelihood ratio criterion. When none of the unselected variables meet the entry criterion, the stepwise selection stops. Variables are chosen to enter or leave the model according to the significance level of an F test. The F test is used to assess whether or not the addition of any specific independent variable to the model significantly improves the prediction of group membership, given that all the other variables are already in the model (Costanza and Afifi, 1979).

We next used the SAS DISCRIM procedure to compute a linear discriminant function and then used it to classify the 20 reference APD into two groups on the basis of the pK_i values for the 5-HT_2, D-1 and D-2 binding sites. The discriminant function, also known as a classification criterion, is determined by a measure of generalized squared distance (Rao, 1973). The classification criterion was then applied to the group of 17 test compounds which are less certainly classified as typical or atypical to reexamine the relationships between pK_i values and type of antipsychotic previously examined for the 20 reference APDs. The univariate and multivariate analyses carried out for the reference APDs were then repeated for all 37 compounds. A preliminary report of this study with fewer APDs has appeared elsewhere (Matsubara and Meltzer, 1988).

Finally, we used the SAS CLUSTER procedure to determine the number and nature of the optimal number of clusters for all 37 compounds using only the 5-HT_2/D-2 ratio. The results are reported utilizing Ward's minimum variance method (Ward, 1963).

Results

The pK_i values for D-1, D-2 and 5-HT_2 binding sites for the 13 typical and 7 atypical reference APDs are given in table 1, along with the ratios of the pK_i values: D-1/D-2, 5-HT_2/D-1 and 5-HT_2/D-2. The Hill coefficients, determined by the EBDA-LIGAND program or graphically, for each receptor binding site did not significantly differ from unity, indicating that under these conditions, the compounds bound to a single class of receptor sites (data not presented). As indicated in table 2, the D-2 pK_i values of the typical drugs were significantly greater than those of the atypical drugs. There was a trend in the same direction for the D-1 pK_i values. There was no difference in the pK_i values for the 5-HT_2 binding sites. The 5-HT_2/D-1 and 5-HT_2/D-2 ratios were significantly lower for the typical compared to the atypical APD. There was a trend in the same direction for the D-1/D-2 ratio. There was no overlap in the 5-HT_2/D-2 ratio between the two groups (table 1).

TABLE 1

pK_i values of D-1, D-2 and 5-HT_2 receptor binding sites and ratios for reference typical and typical APDs

Group	pK_i Values			Ratios of pK_i Values		
	D-1	D-2	5-HT_2	D-1/D-2	5-HT_2/D-1	5-HT_2/D-2
Typical						
Chlorpromazine	7.5	8.5	8.7	0.88	1.16	1.02
Trifluoperazine	7.7	8.9	8.4	0.88	1.08	0.95
Fluphenazine	8.3	9.2	8.6	0.90	1.04	0.94
Perphenazine	7.9	9.2	8.6	0.86	1.08	0.93
Prochlorperazine	8.0	9.2	8.2	0.86	1.12	0.89
Haloperidol	7.0	9.0	7.7	0.79	1.09	0.86
Moperone	6.4	8.7	7.7	0.74	1.20	0.89
Pimozide	6.1	9.4	8.1	0.64	1.34	0.86
cis-Flupentixol	8.6	9.0	8.7	0.96	1.01	0.96
Thiothixene	7.5	9.2	7.3	0.81	0.97	0.79
Loxapine	7.5	8.1	8.7	0.94	1.16	1.09
Molindone	5.8	7.8	6.3	0.74	1.09	0.81
(+)-Butaclamol	8.4	9.5	8.5	0.88	1.01	0.89
Atypical						
Clozapine	6.8	7.0	8.3	0.98	1.22	1.19
Fluperlapine	6.8	6.5	8.1	1.04	1.18	1.24
RMI 81582	8.4	7.1	8.6	1.19	1.02	1.21
Metperone	5.6	6.7	7.5	0.84	1.34	1.13
Amperozide	6.2	6.3	8.0	0.99	1.27	1.26
Setoperone	6.0	7.9	9.4	0.77	1.56	1.20
Tiospirone	6.7	8.8	10.2	0.77	1.51	1.15

TABLE 2

pK_i values[a] of D-1, D-2 and 5-HT_2 receptor binding sites of typical and atypical APDs

Binding Sites	Typical (13)	Atypical (7)	P[b]
D-1	7.44 ± 0.89	6.67 ± 0.90	.080
D-2	8.89 ± 0.51	7.18 ± 0.87	.0001
5-HT_2	8.11 ± 0.71	8.57 ± 0.91	.23
D-1/D-2	0.84 ± 0.09	0.94 ± 0.16	.080
5-HT_2/D-1	1.10 ± 0.10	1.30 ± 0.19	.005
5-HT_2/D-2	0.91 ± 0.08	1.20 ± 0.05	.0001

[a] Mean ± S.D.
[b] As determined by analysis of variance.

TABLE 3

Correlations between pK_i values for D-1, D-2 and 5-HT_2 receptor binding sites reference typical and atypical drugs

Antipsychotics	n	Spearman Correlation of pKi Ratios		
		D-1-D-2	5-HT_2-D-1	5-HT_2-D-2
All reference compounds	20	0.47**	0.41†	0.12
Typical	13	0.37	0.61**	−0.09
Atypical	7	0.11	0.32	0.89**

* $P < .05$; ** $P \leq .07$; † $P < .10$.

Table 3 provides the correlations between pK_i values for all 20 reference APDs and for the 13 typical and 7 atypical APDs separately. A significant correlation between D-1 and D-2 pK_i values was present for the entire group but fails to achieve statistical significance for either subgroup alone. There was a trend for the 5-HT_2 and D-1 pK_i values to be correlated for all 20 compounds. This was significant only for the 13 typical compounds. The greatest difference between the two groups was for the 5-HT_2-D-2 correlations. A highly significant 5-HT_2-D-2 correlation was found for the 13 atypical APDs (rho = 0.89, P = 0.007) compared to none for the typical APD (rho = −0.09).

The stepwise discriminant function determined that the D-2

pK_i value should be entered first because it accounted for the largest portion of the variance, 64%, then the 5-HT_2 affinity which accounted for another 17% of the variance (table 4). The D-1 pK_i value did not meet the criterion for entry ($P = 0.10$). The average squared canonical correlation is the maximal multiple correlation and provides an estimate of the variance accounted for by the D-2 and 5-HT_2 pK_i values.

We next carried out a discriminant function analysis using the D-1, D-2 and 5-HT_2 pK_i values. The discrimination of the two groups of APDs based on these parameters was highly significant (table 5). Examination of the standardized discriminant coefficients indicates the order of contribution to the discriminant function was D-2 > 5-HT_2 ≫ D-1. The relative magnitude and sign of the standardized discriminant coefficients provides an index of the contribution of each type of pK_i value to the discriminant function. Thus, together with the class means presented in table 5, it was concluded that the low D-2 pK_i values of atypical drugs contribute more than their high 5-HT_2 pK_i values and much more than their low D-1 pK_i values to the discrimination from typical APDs.

The linear discriminant function was applied to the 20 reference compounds and the 17 test compounds. The pK_i values of the 17 test compounds are given in table 6. The Hill coefficients of these 17 compounds for each of the receptor binding sites also did not differ significantly from unity. The raw canonical correlations from the discriminant function provide the coefficients for the linear discriminant function (Y) to classify the test compounds. Table 7 provides a list of the compounds as classified on the basis of this equation which is given in the table. A completely symmetrical cutoff point was used to classify the compounds (Kleinbaum and Kupper, 1978). The cutoff point, 6.48, was based on the average of the mean

TABLE 4

Stepwise discriminant function for reference typical and atypical drugs

	F	P	Averaged squared canonical correlation
First variable entered: pK_i D-2	31.33	0.0001	0.64
Second variable entered: pK_i 5-HT_2	15.23	0.001	0.81
Significance level to enter = .10			

TABLE 5

Discriminant analysis with variables pK_i of D-1, D-2 and 5-HT_2 receptor binding sites for reference typical and atypical APDs

The class means for each group were obtained by the following procedure. First, the mean of the pK_i value for all 20 compounds for each binding site was determined and subtracted from that of each drug to obtain three centralized pK_i values for each drug. Next, each of these centralized pK_i values was multiplied by the respective unstandardized discriminant coefficients for the corresponding pK_i value given in the equation in table 7. This produces a canonical score for each drug. The class mean is the mean canonical score for each group. The magnitude and sign of the class means provide a measure of how well the two groups are separated.

Variable	Standardized Discriminant Coefficient
pK_i D-1	0.49
pK_i D-2	2.05
pK_i 5-HT_2	−1.22
Class Means on Canonical Variables	
Type	**Class Mean**
Atypical	−2.89
Typical	1.56

TABLE 6

pK_i values of D-1, D-2 and 5-HT_2 receptor binding sites and ratios for test typical and atypical APDs

Compound	pK_i Values			Ratios of pK_i Values		
	D-1	D-2	5-HT_2	D-1/D-2	5-HT_2/D-1	5-HT_2/D-2
Typical						
Thioridazine	7.5	8.1	8.2	0.93	1.10	1.02
Mesoridazine	7.3	8.1	8.2	0.90	1.13	1.01
Amoxapine	7.2	7.7	8.9	0.93	1.23	1.15
Clothiapine	8.1	8.7	9.2	0.93	1.14	1.06
Methiothepin	8.7	9.7	9.4	0.90	1.08	0.97
Spiperone	5.7	10.0	9.4	0.57	1.65	0.94
Benperidol	7.9	9.7	8.6	0.82	1.08	0.89
Atypical						
Ritanserin	7.0	7.9	9.7	0.89	1.39	1.23
Pipamperone	6.0	6.9	9.2	0.88	1.53	1.34
Rilapine	8.5	7.4	9.1	1.15	1.07	1.24
Tenilapine	7.7	5.8	7.4	1.32	0.96	1.26
SCH 23390	9.6	6.2	7.7	1.55	0.81	1.26
Perlapine	6.6	6.3	7.9	1.05	1.19	1.26
Zotepine	8.6	9.0	9.2	0.96	1.06	1.02
CGS 10746	6.0	5.2	5.8	1.15	0.98	1.12
HP 370	7.9	8.1	8.1	0.98	1.03	1.01
FG 5803	5.4	6.3	8.0	0.86	1.48	1.27

TABLE 7

Classification analysis of 37 putative APDs based on pK_i values for D-1, D-2 and 5-HT_2 receptor binding sites of 20 reference drugs

Equation to classify antipsychotic drugs:

$$\text{Log } Y = 0.520 \times pK_i \text{ D-1} + 1.952 \times pK_i \text{ D-2} - 1.544\ pK_i \text{ 5-HT}_2$$

$$\text{Cutoff Point} = \tfrac{1}{2}(\text{Log } Y_{typ} + \text{Log } Y_{atyp}) = 6.48$$

Typical	Log Y Score	Atypical	Log Y Score
Thiothixene	10.64	Loxapine*	6.15
Butaclamol	9.88	Amoxapine*	5.10
Benperidol	9.82	SCH 23390	5.03
Prochlorperazine	9.45	RMI-81582	5.00
Haloperidol	9.28	Tiospirone	5.00
Pimozide	8.98	Rilapine	4.72
Methiothepin	8.95	Clozapine	4.36
Perphenazine	8.89	Melperone	4.22
Fluphenazine	8.84	CSG 10746	4.22
cis-Flupentixol	8.62	Tenilapine	4.02
Molindone	8.55	Ritanserin	3.99
Moperone	8.38	Setoperone	3.96
Trifluoperazine	8.33	Fluperlapine	3.84
Spiperone	7.98	Perlapine	3.52
Zotepine*	7.83	Amperozide	3.25
HP-370*	7.30	FG-5803	2.73
Chlorpromazine	7.08	Pipamperone	2.32
Clothiapine	6.93		
Thioridazine	6.92		
Mesoridazine	6.88		

* Incorrectly classified.

Y scores of the typical and atypical groups as indicated in table 7. Compounds with scores below 6.48 were considered atypical; those with higher scores, typical. The classification was correct for 34 of 38 (89.5%) compounds. Two of the 17 (11.8%) atypical (zotepine and HP 370) and two of 20 (10%) typical (loxapine and amoxapine) APDs were misclassified. Thioridazine and mesoridazine were classified as typical drugs, although their log Y scores were the lowest of any typical compounds.

The univariate and multivariate analyses described previ-

ously for the reference compounds were repeated for the entire group of 37 compounds using the classification based on the preclinical and clinical data. Thioridazine and mesoridazine were classified as typical compounds. No significant difference in pK_i values for D-1 binding sites was found for the combined group of reference and test drugs (typical = 7.46 ± 0.89, n = 20; atypical = 7.05 ± 1.20, n = 17). The pK_i values for the D-2 binding sites for the typical (8.87 ± 0.67) and atypical drugs (7.01 ± 1.03) were still highly significantly different (P = 0.001). There again was no difference in 5-HT_2 pK_i values between the typical and atypical APDs (8.37 ± 0.73 *vs.* 8.36 ± 1.03, respectively). The correlations between the pK_i values for the D-1, D-2 and 5-HT_2 binding sites for the larger series of compounds are given in table 8. Noteworthy changes from the reference APDs include the findings that the correlation between 5-HT_2 and D-1 pK_i values for all 37 compounds was nonsignificant but the 5-HT_2-D-2 correlation was now significant. The D-1-D-2 pK_i value correlation for all compounds and the 5-HT_2-D-1 pK_i value correlations for the typicals and atypicals were less robust. The stepwise discriminant function entered the D-2 pK_i value first (F = 43.98, P = 0.0001, average squared canonical correlation = 0.56) and then the 5-HT_2 value (F = 14.81, P = 0.0005, average squared canonical correlation = 0.69). Unlike the case with the 20 reference APDs, the pK_i values for the D-1 binding site for all 37 compounds could not be entered (F = 0.078, P = 0.78). The same four compounds were misclassified. The classification based on all 37 compounds was the same as that based on the 20 reference APDs (data not presented).

Based only on the 5-HT_2/D-2 ratio, a cluster analysis yielded two main clusters. One cluster had 19 of 20 typical compounds from tables 1 and 6, plus the atypical compounds HP-370 and zotepine. Amoxapine was clustered along with 15 of 17 atypical compounds (all except HP-370 and zotepine) from tables 1 and 6 in the second cluster. Thus, 35 of 38 (91.9%) of the compounds were clustered in conformity with the preclinical and clinical extrapyramidal or endocrine data simply on the basis of the ratio of the pK_i values for the 5-HT_2 and D-2 receptor sites. The ratio ranged from 0.79 to 1.09 in the cluster with typical compounds and from 1.12 to 1.43 in the cluster with mainly atypical compounds. The error rates for the clusters based on the pK_i values for the 5-HT_2 and D-2 receptor binding sites separately was 13% compared to a 34% error rate for that based on the ratio of the pK_i values for the D-1 and D-2 receptor binding sites. The error rate for the clusters based on the pK_i values for the 5-HT_2, D-1 and D-2 receptor binding sites was 29%.

Discussion

The major findings of this study are that the typical and atypical APDs can be distinguished from each other on the basis of lower D-2 and higher 5-HT_2 pKi values of the atypical compounds as revealed by multivariate analysis. D-1 pK_i values contributed very slightly to the discrimination between the two groups of drugs. We also demonstrated significant univariate differences between the two classes of compounds with regard to D-2 but not 5-HT_2 values. A difference in D-1 pK_i values between the two types of drugs was found for the 20 reference APDs but not the larger group of 37 compounds. A highly significant correlation between 5-HT_2 and D-2 pK_i values for all atypical compounds was noted. Atypical and typical APDs form two clusters based on the 5-HT_2/D-2 ratio only.

The basis for choosing the 20 reference APDs was clinical evidence that the typical APDs produce EPS, TD or large serum PRL increases whereas the atypical APDs produce minimal EPS, no or minimal increases in serum PRL levels in humans, or both (Meltzer and Fang, 1976; Langer *et al.*, 1976; Robertson *et al.*, 1982). The typical APDs also produce potent catalepsy in rodents at doses that are not highly sedative, whereas the atypicals do not (Burki, 1975; Schmutz and Picard, 1980; Baldessarini, 1985; Janssen and Van Bever, 1978). There is ambiguity about the catalepsy potential of some APDs, mainly because of differences in the method of determination (Schmutz and Picard, 1980; Sanberg *et al.*, 1988). There is clinical evidence of low EPS for all seven reference atypical compounds. Fluperlapine (Woggon *et al.*, 1984, 1985), RMI 81582 (Young and Meltzer, 1980), ritanserin (Gelders *et al.*, 1986), melperone (Bjerkenstedt *et al.*, 1978), tiosperone (Bristol-Myers Investigators Brochure), amperozide (R. Axelsson, personal communication 11/4/88) and setoperone (Ceulemans, 1985) have all been shown to produce lower EPS and lower serum PRL levels in humans than typical neuroleptics. Melperone, like clozapine, has had extensive, prolonged clinical use and has never been shown to produce TD (A. Bjork, personal communication, 4/1/89). There is also supportive animal evidence that RMI 81582, like clozapine, did not cause dyskinesias in haloperidol-primed monkeys (Neale *et al.*, 1983). Tiospirone, given p.v., does not produce catalepsy in rats (Bristol-Myers Investigator Manual).

As indicated, assignment of the test compounds to each drug class is less certain. Only six: thioridazine, mesoridazine, amoxapine, ritanserin, perlapine and zotepine have been tested in humans and can be classified on the basis of the clinical criteria used for the reference compounds. The classification of thioridazine is particularly difficult as there is so much controversy about this issue. Thioridazine (and its metabolite mesoridazine) produce low EPS, probably due to their anticholinergic properties and, for that reason, we (Gudelsky and Meltzer, 1989), like other investigators (Altar *et al.*, 1986b, 1988) have previously considered both compounds to be atypical. However, thioridazine produces large increases in serum PRL levels in humans (Sachar *et al.*, 1975; Meltzer and Fang, 1976) as well as TD (Cole and Gardos, 1976) and can cause catalepsy in rats (Kreiskott, 1980). Mesoridazine, the major metabolite of thioridazine, also produces large increases in serum PRL levels in humans (H. Y. Meltzer, unpublished data). Moreover, the clinical difference between clozapine and thioridazine is exemplified by the report that clozapine does not worsen EPS in L-dopa-treated Parkinson's patients but thioridazine, even at low doses (75 mg/day), like haloperidol, produced severe akinesia in these patients (Scholz and Nichgans, 1985). In humans, molindone produces the same degree of EPS (Clark *et al.*, 1970) and increases in plasma PRL levels (H. Y. Meltzer, unpublished

TABLE 8

Correlations between pK_i values for D-1, D-2 and 5-HT_2 receptor binding sites for all typical and atypical compounds

Antipsychotics	n	Spearman Correlations of pK_i Ratios		
		D-1-D-2	5-HT_2-D-1	5-HT_2-D-2
All compounds	37	0.35*	0.27	0.37*
Typical	20	0.31	0.40†	0.15
Atypical	17	0.32	0.18	0.85**

Statistical significance is represented by the following symbols: * P < .05; ** P = .0001; † P = .08.

observations) as do typical neuroleptic drugs. Therefore, we classified thioridazine, mesoridazine and molindone as typical compounds. Amoxapine also produces typical EPS and PRL elevations (Robertson *et al.*, 1982). Ritanserin (Bersani *et al.*, 1986) and perlapine (Stille *et al.*, 1973) are classified as atypical by virtue of their low EPS. Zotepine produces a significantly lower incidence of EPS compared to other neuroleptic drugs (Yamawaki, 1987). However, its antidopaminergic properties in rodents, including the induction of catalepsy, are equivalent to chlorpromazine (Yamawaki, 1987). On the basis of the human data, we classified it as an atypical compound.

The following compounds produce no or weak catalepsy or slight PRL stimulation in rodents and were therefore considered to be atypical: amperozide (Gustafsson *et al.*, 1986); pipamperone (Janssen and Van Bever, 1978; Meltzer *et al.*, 1983), ritanserin (Goldstein *et al.*, 1987; Goldstein and Litwin, 1988), perlapine (Burki *et al.*, 1975; Wilk and Stanley, 1977), SCH 23390 (Iorio *et al.*, 1983; Clark and White, 1988; but see Christensen *et al.*, 1984), tenilapine and rilapine (Knoll Preclinical Research Summary, Knoll Pharmaceuticals), FG 5803 (Ferrosan New Product Manual), HP 370 (Szewczak *et al.*, 1987) and CGS 10746B (Altar *et al.*, 1986a). On the other hand, amoxapine (Greenblatt *et al.*, 1978; Jue *et al.*, 1982), clothiapine (Costall *et al.*, 1975) and methiothepin (Metysova, 1968; Protiva, 1977), spiperone and benperidol (Janssen and Van Bever, 1978), all produce marked catalepsy in rodents and were therefore classified as typical APDs.

The pK_i values reported here for D-1, D-2 and 5-HT_2 binding sites are in good agreement with those in the literature (*e.g.*, Altar *et al.*, 1986b; Andersen *et al.*, 1986; Peroutka and Snyder, 1980). All the Hill coefficients were not significantly different from unity, indicating that under the conditions of these experiments, the ligands were binding to single sites. There were no significant univariate differences between pK_i values for 5-HT_2 and D-1 binding sites between the entire groups of typical and atypical antipsychotic compounds. However, as discussed subsequently, the 5-HT_2 affinities do differ once the D-2 affinities are controlled for by multivariate techniques. The markedly lower affinity of the atypical APD for D-2 receptor binding sites *in vitro* in both the univariate and multivariate analyses in noteworthy. It suggests that D-2 affinity *per se* may be an important factor in determining if an APD will be typical or atypical. The D-2 pK_i values of 18 of 20 typical drugs was $\geq$ 8.1; the only typical drugs with lower pK_i values were molindone and amoxapine. Some of putative atypical compounds had quite low D-2 pK_i values, *e.g.*, CGS 10746B (5.2) and tenilapine (5.8), whereas those of two other atypical drugs, zotepine (9.0) and tiospirone (8.8) were quite high. However, it is noteworthy that zotepine was classified as typical by the model proposed here. This is because its 5-HT_2 pK_i value was only slightly greater than its D-2 pH_i value, producing a 5-HT_2/D-2 ratio of 1.02. Despite its low pK_i value, CGS 10746B blocked apomorphine-induced stereotypy in mice at a dose of 6.6 mg/kg i.p. (Altar *et al.*, 1986b). Similarly, tenilapine is more effective than chlorpromazine in inhibiting the conditioned avoidance response in rats (ED_{50}: 6.8 mg/kg p.o. *vs.* 11.6 mg/kg) and was more potent than chlorpromazine in blocking the effect of apomorphine on turning in rats with unilateral substantia nigra lesions (Preclinical Research Summary, Knoll Pharmaceuticals). These findings suggest functional D-2 receptor blockade may not be necessary for some atypical drugs to produce their antipsychotic effect or to block some of the effects of DA agonists. Fardé *et al.* (1989) have shown that at clinically efficacious doses, clozapine produces a lower occupancy of striatal D-2 receptors than APDs. If these results are confirmed in a larger group of subjects, and if it also applies to the mesocorticolimbic D-2 receptors, it would suggest D-2 receptor blockade cannot account for the increased antipsychotic efficacy of clozapine.

Because there were significant, albeit differing, correlations among the pK_i values for the three binding sites within the two groups of APDs, it is necessary to adjust for these correlations by multivariate statistical techniques to assess the independent influence of each of the pK_i values for determining class membership. The stepwise discriminant function indicated that the pK_i value for the D-2 and 5-HT_2 receptor binding sites contribute most to the classification of an antipsychotic compound as atypical. The D-1 pK_i value did not meet the criterion for entry ($P < .15$) into the model. These results provide a possible explanation why *cis*-flupentixol, (+)-butaclamol and methiothepin, which have high affinities for the D-1 binding site, but whose pK_i values for the D-2 and 5-HT_2 binding sites are such that the 5-HT_2/D_2 ratio is not in the range of that of the atypical drugs reported in tables 1 and 6, lack atypical properties. Drugs such as SCH 23390 which have relatively low D-2 and 5-HT_2 pK_i values but with a 5-HT_2/D_2 ratio in the 1.10 to 1.27 range may be atypical for that reason and not because of their high D-1 affinity. The 5-HT_2 antagonist properties of SCH 23390 have been pointed out by Bischoff *et al.* (1988), but they suggested that this effect might be minimal at doses which achieve D-1 receptor blockade. A recent study demonstrated a correlation of 0.84 between the K_i value for D-1 and 5-HT_2 binding sites for a series of 17 compounds related to SCH 23390 (McQuade *et al.*, 1988). These compounds, like SCH 23390, had a greater affinity for the D-1 than the 5-HT_2 binding site. However, *in vivo* binding studies demonstrated effective blockade at both the D-1 and 5-HT_2 receptor sites.

The discriminant function analysis confirmed the importance of the pK_i values for the D-2 and 5-HT_2 binding sites for classifying the compounds under study as typical or atypical. The magnitude of the standardized canonical correlations provides an estimate of the independent contribution of the pK_i value for each binding site to the model. The discriminant function correctly identified all but one of the reference compounds (loxapine) and 14 of 17 test compounds, erring with amoxapine, zotepine and HP-370. It should be noted that the classification analysis, although based on the linear function developed from the reference compounds, need not classify these compounds as originally used to develop the function and, in fact, identified loxapine as an atypical compound, although it was just on the borderline between typical and atypical compounds (table 7).

The mistakes in classification by the discriminant function appear to be related to the importance of the 5-HT_2/D-2 ratio for class assignment. Loxapine and amoxapine were classified as atypical but all available clinical evidence with these APDs indicates they are typical neuroleptics (Tuason *et al.*, 1984; Robertson *et al.*, 1982). The ratios of pK_i values for 5-HT_2 and D-2 binding sites for amoxapine (1.15) and loxapine (1.09) are near the lowest value (melperone: 1.13) of the reference atypical APDs. Conversely, the 5-HT_2/D-2 ratio for the other two erroneously classified drugs, HP-370 (1.01) and zotepine (1.02), are close to that of chlorpromazine, thioridazine and clothiapine, the typical drugs with the highest ratios. Zotepine does produce moderate-marked catalepsy in rats (Uchida *et al.*, 1979;

Yamawaki, 1987) and might have been classified as a typical APD on the basis of the preclinical data. It clearly has potent antiserotonergic properties *in vivo* (Yamawaki, 1987). Further studies are indicated to determine whether the low EPS it produces in patients is related to its antiserotonergic effects or some other mechanism.

Thioridazine and mesoridazine were both classified as typical APDs. Their 5-HT_2/D-2 ratios, 1.02 and 1.01 of these two drugs, were well within the group of typical APDs. However, their log Y scores were the closest to those of the atypical APDs of any of the typical compounds. This suggests a small contribution of D-1 affinities to their overall clinical effects. The borderline nature of these APDs may explain why these compounds behave like atypical APDs in some test conditions (Altar *et al.*, 1986b, 1988; Gudelsky and Meltzer, 1989). Clinically, they behave like typical APDs with regard to PRL secretion and TD, but like atypical APDs, with regard to EPS.

The hypothesis that a critical balance between effects on dopaminergic and serotonergic mechanisms is essential for an APD to be atypical is supported by the high correlation between D-2 and 5-HT_2 affinities for the atypical compounds. It suggests that balanced changes in dopaminergic and serotonergic function *in vivo*, based mainly on D-2 and 5-HT_2 receptor antagonism, are necessary if a drug is to be an atypical APD. Absolute affinities for D-2 and 5-HT_2 binding sites are apparently not of critical importance. The pK_i values of the atypical APDs for the D-2 receptor, although relatively low compared to those of the typical APDs, nevertheless differ by over two orders of magnitude within each group (table 1). However, when accompanied by affinities for the 5-HT_2 receptor which lead to ratios of pK_i values of 5-HT_2 and D-2 binding sites of 1.15 or greater, atypical APD properties will result. Low D-2 values, if associated with high 5-HT_2/D_2 ratios, are permissible for atypical APDs. Both fluperlapine (Woggon *et al.*, 1984) and amperozide (R. Axelsson, personal communication, 11/4/88) are clearly antipsychotic despite low affinities for the D-2 receptor (287 and 495 nM, respectively). Furthermore, the antipsychotic dose range of these two drugs (10–20 mg/day for amperozide and 200–500 mg/day for fluperlapine) are far less than would be predicted on the basis of their very weak D-2 potency. Thus, the long held belief that there is an extremely close relationship between the dose of all APDs which are D-2 antagonists and affinity for the [^{3}H]haloperidol (Seeman *et al.*, 1976) or [^{3}H] spiperone binding site (Peroutka and Snyder, 1980) may no longer be tenable.

Absolute potency as a 5-HT_2 antagonist alone also cannot be the decisive factor for determining whether an APD has atypical properties. The pK_i value of clozapine for the cortical 5-HT_2 binding site is 8.33. Spiperone, clothiapine, chlorpromazine, trifluoperazine and fluphenazine, all of which are typical APDS, have higher 5-HT_2 pK_i values. Furthermore, at least one atypical APD, CGS 10746B, has a very low 5-HT_2 value (table 6). Based upon the results reported here, it seems more likely that the 5-HT_2 affinity is important for conveying atypical properties mainly in relation to the D-2 affinity.

The importance of the 5-HT_2/D-2 ratio is confirmed by the results of the cluster analysis. The optimal number of clusters formed was two and these were virtually identical with the groups formed on the basis of the ability to produce EPS or PRL stimulation in humans or catalepsy in rodents. There was no overlap in 5-HT_2/D-2 ratios between the two clusters. In the future, it will be of interest to determine if other compounds which fall within these clusters have comparable typical and atypical properties.

It is possible that the relationship between *in vitro* affinity for 5-HT_2 and D-2 receptor binding sites and atypical APD status reported here is unrelated to important physiological effects on D-2 and 5-HT_2 receptors *in vivo*, *i.e.*, that these characteristics are predictive of some other biological feature(s) that are the critical elements in conveying atypical properties on these APDs. However, there is considerable evidence of modulatory effects of the serotonergic system of dopaminergic mechanisms, and *vice-versa*, which make it possible to consider that there are important physiological consequences of the correlated D-2 and 5-HT_2 antagonist properties of the atypical drugs. The functional importance of D-2 receptor blockade for the antipsychotic, EPS, and PRL-elevating effects of neuroleptic drugs is undisputed (Meltzer and Stahl, 1976). The functional importance of an action of neuroleptic drugs on the serotonergic system is less clear but there is evidence of its relevance, at least in regard to motor aspects of striatal and limbic function (Kostowski *et al.*, 1972; Costall *et al.*, 1975; Carter and Pycock, 1978). Serotonin receptor antagonists diminish, whereas 5-HT agonists or uptake inhibitors enhance, neuroleptic-induced catalepsy (Waldmeier and Delini-Stula, 1979; Balsara *et al.*, 1979). These results suggest that at a functional level at least, diminishing serotonergic activity can decrease the inhibitory effects of neuroleptics on dopaminergic output. This includes the nucleus accumbens, a component of the limbic system generally thought to play a key role in the antipsychotic effects of neuroleptic drugs (Crow *et al.*, 1977). It is unclear whether supplemental addition of a 5-HT_2 antagonist to treatment with a D-2 antagonist such as haloperidol which has weak 5-HT_2 antagonist properties would lead to increased efficacy and fewer EPS. It has been reported recently that the addition of cyproheptadine, a nonspecific 5-HT antagonist, to haloperidol-treated schizophrenic patients, led to improvement in both positive and negative symptoms (Silver *et al.*, 1989).

It is beyond the scope of this paper to discuss the numerous possible ways in which DA and 5-HT might interact to produce normal output of the mesolimbic and mesocortical DA systems. However, one particularly interesting possibility that could explain the importance of a correlated antagonism of D-2 and 5-HT_2 receptors is worth noting here. It is possible that the antiserotonergic properties of atypical APDs might increase dopaminergic activity in the striatum as well as in the frontal cortex. Hervé *et al.* (1981) demonstrated that lesions of the median raphé increased DA turnover in the frontal cortex. The 5-HT_2 antagonist properties of clozapine might produce an effect of the same type. Clozapine administration has been shown to increase DA release in the frontal cortex (Imperato and Angelucci, 1988). The D-2 antagonist properties of clozapine might prevent any psychotomimetic effects of increased dopaminergic activity in the mesolimbic and mesocortical DA systems. Increasing DA activity in the frontal cortex may contribute to improvement in negative symptoms in schizophrenia (Meltzer, 1985). Increasing dopaminergic activity in the frontal cortex might actually lead to decreased dopaminergic activity in the striatum and mesolimbic system (Pycock *et al.*, 1980; Scatton *et al.*, 1982).

It would seem unlikely that so simplistic a concept as the ratio of the affinities for 5-HT_2 and D-2 receptors is the entire explanation of all the unique features of the atypical APD. Effects on 5-HT release (Maidment and Marsden, 1987;

Drescher and Hetey, 1988), ability to enhance DA release (Gudelsky *et al.*, 1987; Imperato and Angelucci, 1988), anticholinergic (Miller and Hiley, 1974), anti-*alpha* adrenergic (Burki *et al.*, 1975; Cohen and Lipinski, 1986) and *beta* adrenergic blockade (Gross and Schümann, 1982), ability to increase γ-aminobutyric acid turnover in the striatum and decrease it in the substantia nigra (Maggi *et al.*, 1977) ability to enhance glutamatergic transmission (Schmidt, 1986) and stimulation of neurotensin release (Kilts *et al.*, 1988; Gudelsky *et al.*, 1989) may also contribute, along with yet other effects, to the spectrum of action of clozapine and perhaps other atypical APDs. It may also be the 5-HT_2/D-2 ratios is relevant only to EPS and TD liability and not to increased antipsychotic efficacy.

In summary, the pK_i values of 38 typical and atypical APD for 5-HT_2, D-1 and D-2 binding sites in rat brain have been determined. Multivariate analytic techniques demonstrate the importance of the D-2 and 5-HT_2 affinities for determining whether a drug which can block the conditioned avoidance response will have atypical properties as an antipsychotic. A 5-HT_2/D-2 ratio $\geq$ 1.12 is characteristic of the atypical APD. These results may be useful in drug screening programs.

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Note Added in Proof

There is some evidence that zotepine may, in fact, produce significant EPS in man (F. Holsboer, personal communication, Aug. 29, 1989).

References

ALTAR, C. A., BOYAR, W. C., WASLEY, A., LIEBMAN, J. M., WOOD, P. L., AND GERHARDT, S. G.: Dopamine neurochemical profile of atypical antipsychotics resembles that of D-1 antagonists. Naunyn-Schmiedeberg's Arch. Pharmacol. **338:** 162–168, 1988.

ALTAR, C. A., WASLEY, A. M., LIEBMAN, J., GERHARDT, S. G., KIM, H., WELCH, J. J. AND WOOD, P. L.: CSG 10746B: An atypical antipsychotic candidate that selectively decreases dopamine release at behaviorally effective doses. Life Sci. **39:** 688–705, 1986a.

ALTAR, C. A., WASLEY, A. M., NEALE, F. F. AND STONE, G. A.: Typical and atypical antipsychotic occupancy of D-2 and S-2 receptors: An autoradiographic analysis in rat brain. Brain Res. Bull. **16:** 517–525, 1986b.

ANDERSEN, P. H. AND BRAESTRUP, C.: Evidence for different states of the dopamine D-1 receptor: clozapine and fluperlapine may preferentially label an adenylate cyclase-coupled state of the D-1 receptor. J. Neurochem. **47:** 1822–1831, 1986.

ANDERSEN, P. H., NIELSEN, E. B., GRONVALD, F. C. AND BRAESTRUP, C.: Some atypical neuroleptics inhibit [^{3}H]SCH 23390 binding *in vivo*. Eur. J. Pharmacol. **120:** 143–144, 1986.

BALDESSARINI, R. J.: Drugs and the treatment of psychiatric disorders. *In* The Pharmacological Basis of Therapeutics, 7th ed., ed. by A. G. Gilman, L. S. Goodman, T. W. Rall and F. Murrad, pp. 387–445, McMillan Publishing Company, New York, 1985.

BALSARA, J. J., JAKHAV, J. H. AND CHANDORKAR, A. G.: Effect of drugs influencing central serotonergic mechanisms on haloperidol-induced catalepsy. Psychopharmacology **62:** 67–69, 1979.

BERSANI, G., GRISPINI, A., MORINI, S., PASINI, A., VALDUCCI, M. AND CIANI, A.: Neuroleptic-induced extrapyramidal side effects: Clinical perspectives with ritanserin (R 55667), a new selective 5-HT_2 receptor blocking agent. Curr Ther Res **40:** 492–499, 1986.

BILLARD, W., RUPERTO, V., CROSBY, G., IORIO, L. C. AND BARNETT, A.: Characterization of the binding of ^{3}H-SCH 23390, a selective D-1 receptor antagonist ligand, in rat striatum. Life Sci. **35:** 1885–1893, 1984.

BISCHOFF, S., HEINRICH, M., KRAUSS, J., SILLS, M. A., WILLIAMS, M. AND VARSOUT, A.: Interaction of the D_1 receptor antagonist SCH 23390 with the central 5-HT system: Radioligand binding studies, measurements of biochemical parameters and effects on L-5-HTP syndrome. J. Receptor Res. **8:** 107–120, 1988.

BJERKENSTEDT, L., HÄRNRYD, C., GRIMM, V., GULLBERG, B. AND SEDVALL, B.: A double-blind comparison of melperone and thiothixene in psychotic women using a new rating scale, the CPRS. Arch. Psychiatr. Nervenkranz. **226:** 157–172, 1978.

BURKI, H. R., RUCH, W. AND ASPEN, H.: Effects of clozapine, thioridazine, perlapine and haloperidol on the metabolism of the biogenic amines in the brain of the rat. Psychopharmacol. (Berl.) **41:** 27–33, 1975.

CARTER, C. J. AND PYCOCK, C. J.: A study of the sites of interaction between dopamine and 5-hydroxytryptamine for the production of fluphenazine-induced catalepsy. Naunyn-Schmideberg's Arch. Pharmacol. **304:** 135–139, 1978.

CEULEMANS, D. L. S.: Effect of serotonin antagonism in schizophrenia: A pilot study with setoperone. Psychopharmacol. **85:** 329–332, 1985.

CHIPKIN, R. E. AND LATRANYI, M. B.: Similarity of clozapine and SCH 23390 in reserpinized rats suggest a common mechanism of action. Eur. J. Pharmacol. **136:** 371–375, 1987.

CHRISTENSEN, A.V., ARNT, J., HYTTEL, J., LARSEN, J.-J., AND SVENDSEN, O.: Pharmacological effects of a specific dopamine D-1 Antagonist SCH 23390 in Comparison with Neuroleptico Life Sci. **34:** 1529–1540, 1984.

CLAGHORN, J., HONIGFELD, G., ABUZZAHAB, F. S., WANG, R., STEINBOOK, R., TUASON, V. AND KLERMAN, G.: The risks and benefits of clozapine versus chlorpromazine. J. Clin. Psychopharmacol. **7:** 377–384, 1987.

CLARK, D. AND WHITE, F. J.: D-1 dopamine receptor—The search for a function: A critical evaluation of the D-1/D-2 dopamine receptor classification and its functional implications. Synapse **1:** 347–380, 1988.

CLARK, M. L., HUBER, W. K., SAKATA, K., FOWLES, D. C. AND SERAFETINIDES, E. A.: Molindone in chronic schizophrenia. Clin. Pharmacol. Ther. **11:** 680–685, 1970.

COHEN, B. M. AND LIPINSKI, J. F.: *In vivo* potencies of antipsychotic drugs in blocking alpha$_1$ noradrenergic and dopamine D-2 receptors: Implications for drug mechanisms of action. Life Sci. **39:** 2571–2580, 1986.

COLE, J. O. AND GARDOS, G.: Psychopharmacology update. J. McLean Hosp. **1:** 155–165, 1976.

COSTALL, B., FORTUNE, D. H., NAYLOR, R. J., MARSDEN, C. D. AND PYCOCK, C.: Serotonergic involvement with neuroleptic catalepsy. Neuropharmacology **14:** 859–868, 1975.

COSTALL, B. AND NAYLOR, R. J.: Neuroleptic interactions with the serotonergic gic-dopaminergic mechanisms in the nucleus accumbens. J. Pharm. Pharmacol. **30:** 257–259, 1978.

COSTANZA, M. C. AND AFIFI, A. A.: Comparison of stopping rules in forward stepwise discriminant analyses. J. Am. Stat. Assoc. **74:** 777–785, 1979.

CROW, T. J., DEAKIN, J. F. W. AND LONGDEN, A.: The nucleus accumbens—Possible site of action of antipsychotic action of neuroleptic drugs. Psycholog. Med. **7:** 213–221, 1977.

DRESCHER, K. AND HETEY, L.: Influence of antipsychotics and serotonin antagonists on presynaptic serotonin receptors modulatory the release of serotonin in synaptosomes of the nucleus accumbens of rats. Neuropharmacology **27:** 31–36, 1988.

FARDÉ, L., NORDSTROM, A.-L., WIESEL, F.-A. AND SEDVALL, G.: D1- and D2-dopamine receptor occupancy during treatment with conventional and atypical neuroleptics. Psychopharmacology, in press, 1989.

FARDÉ, L., WIESEL, F.-A., HALLDIN, C. AND SEDVALL, G.: Central D2-dopamine receptor occupancy in schizophrenic patients treated with antipsychotic drugs. Arch. Gen. Psychiatry **45:** 71–76, 1988.

FINK, H., MORGENSTERN, R. AND OETSSNER, W.: Clozapine—A serotonin antagonist? Pharmacol. Biochem. Behav. **20:** 513–517, 1984.

FJALLAND, B.: Neuroleptic influence on hyperthermia induced by 5-hydroxytryptophan and p-methoxyamphetamine in MAOI-pretreated rabbits. Psychopharmacology **63:** 113–117, 1979.

GELDERS, Y., BUSSCHE, G. V., REYNTJENS, A. AND JANSSEN, P.: Serotonin-S_2 receptor blockers in the treatment of chronic schizophrenia. Clin. Neuropharmacol. **9:** 325–327, 1986.

GOLDSTEIN, J. M. AND LITWIN, L. C.: Antipsychotic like activity of ritanserin following chronic treatment in rats. Fed. Am. Soc. Exp. Biol. J. **2:** A1404, 1988.

GOLDSTEIN, J. M., LITWIN, L. C. AND MALICK, J. B.: Ritanserin increases spontaneous activity of A9 and A10 dopamine neurons. Fed. Proc. **46:** 996, 1987.

GREENBLATT, E. N., LIPPA, A. S. AND OSTERBERG, A. C.: The neuropharmacological actions of amoxapine. Arch. Inter. Pharmacodynamic Ther. **233:** 107–135, 1978.

GROSS, G. AND SCHÜMANN, H. J.: Effect of long term treatment with atypical neuroleptic drugs on beta adrenoceptor binding in rat cerebral cortex and myocardium. Naunyn-Schmiedeberg's Arch. Pharmacol. **321:** 271–275, 1982.

GUDELSKY, G. A., BERRY, S. AND MELTZER, H. Y.: Neurotensin activates tuberoinfundibular dopamine neurons and increases serum corticosterone in the rat. Neuroendocrinology, in press, 1989.

GUDELSKY, G. A., KOENIG, J. I., SIKMONOVIC, M., KOYAMA, T., OHMORI, T. AND MELTZER, H. Y.: Differential effects of haloperidol, clozapine and fluperlapine on tuberoinfundibular dopamine neurons and prolactin secretion in the rat. J. Neural Transm. **68:** 227–240, 1987.

GUDELSKY, G. A. AND MELTZER, H. Y.: Activation of tuberoinfundibular dopamine neurons following the acute administration of atypical antipsychotics. Neuropsychopharmacology **2:** 45–51, 1989.

GUSTAFSSON, B., SVARTENGREN, J. AND CHRISTENSSON, O.: Amperozide—a novel psychotropic compound with selectivity to limbic brain functions. Psychopharmacology **89:** S17, 1986.

HERVÉ, D., SIMON, H., BLANE, G., LEMOAL, M., GLOWINSKI, J. AND TASSIN, J. P.: Opposite changes in dopamine utilization in the nucleus accumbens and the frontal cortex after electrolytic lesions of the median raphé in the rat. Brain Res. **216:** 422–428, 1981.

IMPERATO, A. AND ANGELUCCI, L.: Effect of the atypical neuroleptic clozapine

and fluperlapine on the *in vivo* dopamine release in the dorsal striatum and in the prefrontal cortex. Psychopharmacology **96:** Suppl. 1, Abstract of the XVI CINP Congress, Munich, p. 79, 1988.

IORIO, L. S., BARNETT, A., LEITZ, F. H., HOUSER, V. P. AND KORDUBA, C. A.: SCH 23390, a potential benzazepine antipsychotic with unique interactions on dopaminergic systems. J. Pharmacol. Exp. Ther. **226:** 462–468, 1983.

JANSSEN, P. A. J. AND VAN BEVER, W. F. M.: Structure-activity relationships of the butyrophenones and diphenylbutyl piperdines. *In* Handbook of Pharmacology *10*, ed. by L. L. Iversen, S. D. Iversen and S. H. Snyder, pp. 1–35, Plenum Press, New York, 1978.

JUE, S. G., DAWSON, G. W. AND BROGDEN, R. N.: Amoxapine: A review of its pharmacology and efficacy in depressed states. Drugs **24:** 1–23, 1982.

KANE, J., HONIGFELD, G., SINGER, J., MELTZER, H. Y. AND THE CLOZARIL COLLABORATIVE STUDY GROUP: Clozapine for the treatment-resistant schizophrenic. Arch. Gen. Psychiatry **45:** 789–796, 1988.

KILTS, C. D., ANDERSON, C. M., BISSETTE, G., ELY, T. AND NEMEROFF, C. B.: Differential effects of antipsychotic drugs on the neurotensin concentration of discrete rat brain nuclei. Biochem. Pharmacol. **37:** 1547–1554, 1988.

KLEINBAUM, D. G. AND KUPPER, L. L.: Applied Regression Analysis and Other Multivariate methods; p. 427, Duxbury Press, Belmont, CA, 1978.

KOSTOWSKI, W., GUMULKA, W. AND CZLONKOWSKI, A.: Reduced cataleptogenic effects of some neuroleptics in rats with lesioned midbrain raphé and treated with p-chlorophenylalanine. Brain Res. **48:** 443–446, 1972.

KREISKOTT, H.: Behavioral pharmacology of antipsychotics. *In* Handbook of Experimental Pharmacology, Vol. 55, ed by F. Hoffmeister and G. Stille, pp. 59–89, Springer-Verlag, Berlin, 1980.

LAI, H., CARINO, M. A. AND HOUTA, A.: Antiserontonergic properties of neuroleptic drugs. *In* Psychopharmacology and Biochemistry of Neurotransmitter Receptors. pp. 347–353, Elsevier, New York, 1980.

LANGER, G., SACHAR, E. J., GRUEN, P. H. AND HALPERN, F. S.: Human prolactin responses to neuroleptic drugs correlate with antischizophrenic potency. Nature (Lond.) **266:** 639–640, 1976.

LEYSEN, J. E., NIEMEGEERS, C. J. E., VAN NEUTEN, J. M. AND LADURON, P. M.: ^{3}H-Ketanserin (R 41 468), a selective ^{3}H-ligand for serotonin$_2$ receptor binding sites. Mol. Pharmacol. **21:** 301–314, 1982.

MAGGI, A., CATTABENI, F., BRUNO, F. AND RACAGNI, G.: Haloperidol and clozapine: Specificity of action on GABA in the nigrostriatal system. Brain Res. **133:** 382–385, 1977.

MAIDMENT, N. T. AND MARSDEN, C. D.: Acute administration of clozapine, thioridazine and metoclopramide increase extracellular DOPAC and decreases extracellular 5-HIAA, measured in the nucleus accumbens and striatum of the rat, using *in vivo* voltammetry. Neuropharmacology **26:** 187–193, 1987.

MATSUBARA, S. AND MELTZER, H. Y.: Effect of atypical and typical antipsychotic drugs on D-1 and D-2 dopamine (DA) receptors and serotonin receptors. Soc. Neurosci. Abst. **14:** 370, 1988.

MATZ, R., RICK, W., OH, D., THOMPSON, H. AND GERSHON, S.: Clozapine—A potential antipsychotic agent without extrapyramidal manifestation. Curr. Ther. Res. **16:** 687–695, 1974.

MCQUADE, R. D., FORD, D., DUFFY, R. A., CHIPKIN, R. E., IORIO, L. C. AND BARNETT, A.: Serotonergic compound of SCH 23390: *In vitro* and *in vivo* binding analyses. Life Sci. **43:** 1861–1869, 1988.

MELTZER, H. Y.: Clozapine: Clinical advantages and biological mechanisms. *In* Schizophrenia: A Scientific Focus, ed. by C. Schulz and C. Tamminga, pp. 302–309, Oxford Press, New York, 1989.

MELTZER, H. Y. AND FANG, V. S.: The effect of neuroleptics on serum prolactin levels in schizophrenic patients. Arch. Gen. Psychiatry **33:** 279–286, 1976.

MELTZER, H. Y.: Dopamine and negative symptoms in schizophrenia: Critique of type I-type II hypothesis. *In* Controversies in Schizophrenia: Changes and Constancies, ed. by M. Alpert, pp. 110–136, Guilford Press, New York, 1985.

MELTZER, H. Y., GOODE, D. J., SCHYVE, P. M., YOUNG, M. AND FANG, V. S.: Effect of clozapine on human serum prolactin levels. Am. J. Psychiatry **136:** 1550–1555, 1979.

MELTZER, H. Y. AND LUCHINS, D. J.: Effect of clozapine in severe tardive dyskinesia: A case report. J. Clin. Psychopharmacol. **4:** 286–287, 1984.

MELTZER, H. Y., SIMONOVIC, M. AND GUDELSKY, G. A.: Effects of pirenperone and ketanserin on rat prolactin secretion *in vivo* and *in vitro*. Eur. J. Pharmacol. **92:** 83–89, 1983.

MELTZER, H. Y. AND STAHL, S. M.: The dopamine hypothesis of schizophrenia. Schizophrenia Bull. **2:** 19–76, 1976.

METYSOVA, J.: Pharmacological comparison of a new neuroleptic drug methiothepin with octoclothepin and chlorpromazine. Arch. Nev. Super **10:** 323–333, 1968.

MIKUNI, M., GUDELSKY, G. A., SIMONOVIC, M. AND MELTZER, H. Y.: Interaction of (+)- and (−)-3-PPP with the dopamine receptor in the anterior pituitary gland. Life Sci. **34:** 239–246, 1984.

MILLER, R. J. AND HILEY, C. R.: Anti-muscarinic properties of neuroleptics and drug-induced parkinsonism. Nature (Lond.) **248:** 596–597, 1974.

NASH, J. F., MELTZER, H. Y. AND GUDELSKY, G. A.: Antagonism of serotonin receptor-mediated neuroendocrine and temperature responses by atypical neuroleptics in the rat. *Eur. J. Pharmacol.* **151:** 463–469, 1988.

NEALE, R., GERHARDT, S. AND LIEBMAN, J. M.: Effects of the novel antipsychotics, RMI 81582 and clopipazan, on the predictors of extrapyramidal liability in squirrel monkeys. Drug Dev. Res. **3:** 171–176, 1983.

PEROUTKA, S. J. AND SNYDER, S. H.: Relationship of neuroleptic drug effects at brain dopamine, serotonin, alpha-adrenergic, and histamine receptor to clinical potency. Am. J. Psychiatry **137:** 1518–1522, 1980.

PROTIVA, M.: Metitepine. *In* Drugs of the Future, II, pp. 250–267, 1977.

PYCOCK, C. J., CARTER, C. J. AND KERWIN, R. W.: Effect of 6-hydroxydopamine lesions of the medial prefrontal cortex on neurotransmitter systems in subcortical sites in the rat. J. Neurochem. **34:** 91–99, 1980.

RAO, C. R.: Linear Statistical Reference and Its Application, John Wiley and Sons, New York, 1973.

RASMUSSEN, K. AND AGHAJANIAN, G. K.: Potency of antipsychotics in reversing the effects of a hallucinogenic drug on locus coeruleus neurons correlates with compound binding affinity. Neuropsychopharmacology **1:** 101–107, 1988.

ROBERTSON, A. G., BERRY, R. AND MELTZER, H. Y.: Prolactin stimulating effects of amoxapine and loxapine in psychiatric patients. Psychopharmacology **78:** 287–292, 1982.

RUPNIAK, N. M. J., HALL, M. D., MANN, S., FLEMINGER, S., KILPATRICK, G., JENNER, P. AND MARSDEN, C. D.: Chronic treatment with clozapine, unlike haloperidol, does not induce changes in striatal D-2 receptor function in the rat. *Biochem. Pharmacol.* **34:** 2755–2763, 1985.

SACHAR, E. J., GRUEN, P. H., KARASU, T. B., ALTMAN, N. AND FRANTZ, A. G.: Thioridazine stimulates prolactin secretion in man. Arch. Gen. Psychiatry **32:** 885–886, 1975.

SANBERG, P. R., BUNSEY, M. D., GIORDANO, M. AND NORMAN, A. B.: The catalepsy test. Its ups and downs. Behav. Neurosci. **102:** 748–759, 1988.

SCATTON, B., WORMS, P., LLOYD, K. G. AND BARTHOLINI, G.: Cortical modulation of striatal function. Brain Res. **232:** 331–343, 1982.

SCHMIDT, W. J.: Intrastriatal injection of DL-2-amino-5-phosphonovaleric acid (AP-5) induces sniffing stereotypy that is antagonized by haloperidol and clozapine. Psychopharmacology **90:** 123–130, 1986.

SCHMUTZ, J. AND PICARD, C. W.: Tricyclic neuroleptics: structure-activity relationship. *In* Handbook of Experimental Pharmacology, Vol. 55, ed. by F. Hoffmeister and G. Still, pp. 3–25, Springer-Verlag, New York, 1980.

SCHOLZ, E. AND DICHGANS, J.: Treatment of drug-induced exogenous psychosis in parkinsonism with clozapine and fluperlapine. Eur. Arch. Psychiatr. Neurol. Sci. **235:** 60–64, 1985.

SEEMAN, P.: Brain dopamine receptors. Pharmacol. Rev. **33:** 229–313, 1980.

SEEMAN, P., LEE, T., CHANG-WONG, M. AND WONG, K.: Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature (Lond.) **261:** 717–718, 1976.

SILVER, H., BLACKER, M., WELLER, M. P. I. AND LERER, B.: Treatment of chronic schizophrenia with cyproheptadine. Biol. Psychiatry **25:** 502–504, 1989.

STILLE, G., SAGERS, A. C., LAUENER, H. AND EICHENBERGER, E.: 6-(4-Methyl-1-piperazinyl)-morphantridine (perlapine) a new tricyclic compound with sedative and sleep-promoting properties. Psychopharmacologia (Berl.) **28:** 325–337, 1973.

STOCKMEIER, C. A. AND KELLAR, K. J.: In vivo regulation of the serotonin-2 receptor in rat brain. Life Sci. **38:** 117–127, 1986.

SULPIZIO, A., FOWLER, P. J. AND MACKO, E.: Antagonism of fenfluramine-induced hyperthermia: A measure of central serotonin inhibition. Life Sci. **22:** 1439–1446, 1978.

SZEWCZAK, M. R., CORNFELDT, M. L., DUNN, R. W., WILKER, J. C., GEYER, H. M. III, GRAMKOWSKI, E. J., CHIANG, Y. AND FIELDING, S.: Pharmacological evaluation of HP 370, a potential antipsychotic agent: 1. *In vivo* profile. Drug Develop. Res. **11:** 157–168, 1987.

TUASON, V. B., ESCOBAR, J. I., GARVEY, M. AND SCHIELE, B.: Loxapine versus chlorpromazine in paranoid schizophrenia: A double-blind study. J. Clin. Psychiatry **45:** 158–163, 1984.

UCHIDA, S., HONDA, F., OTSUKA, M., SATOK, Y., MORI, J., ONO, T. AND HITOMI, M.: Pharmacological study of [2-chloro-11-(2-dimethylaminoethoxy)dibenzo-(b,f)-thiepine] (zotepine), a new neuroleptic drug. Arzneim.-Forsch/Drug Res. **29:** 1588–1594, 1979.

WALDMEIER, P. C. AND DELINI, STULA, A. A.: Serotonin-dopamine interactions in the nigrostriatal system. Eur. J. Pharmacol. **55:** 363–373, 1979.

WARD, J. H.: Hierarchial grouping to optimize an objective function. J. Am. Stat. Assoc. **58:** 236–244, 1963.

WILK, S. AND STANLEY, M.: Perlapine and dopamine metabolism: prediction of antipsychotic efficacy. Eur. J. Pharmacol. **41:** 65–72, 1977.

WOGGON, B., HEINREICH, K., KUFFERLE, B., MILLER-OERLINGHAUSER, POLDINGER, W., RUTHER, E. AND SCHIED, H. W.: Results of a multicenter AMPD study with fluperlapine in schizophrenic patients. Arzneim. Forsch. **34:** 122–124, 1984.

WOGGON, B., HEINREICH, K., KUFFERLE, B., MILLER-OERLINGHAUSER, POLDINGER, W., RUTHER, E. AND SCHIED, H. W.: Fluperlapine—A potential successor to clozapine. Pharmacopsychiatry **18:** 73–74, 1985.

YAMAWAKI, S.: Profiles of clinical efficacy and pharmacological action of zotepine. Pharmacopsychiatry **20:** 4–7, 1987.

YOUNG, M. A. AND MELTZER, H. Y.: RMI-81,582, a novel anti-psychotic drug. Psychopharmacology **67:** 101–106, 1980.

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ELSEVIER

The Role of Serotonin in Antipsychotic Drug Action

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Recent interest in the role of serotonin (5-HT) in antipsychotic drug action is based mainly upon the fact that antipsychotic drugs such as clozapine, olanzapine, quetiapine, risperidone, sertindole, and ziprasidone are potent 5-HT_{2a} receptor antagonists and relatively weaker dopamine D_2 antagonists. These agents share in common low extrapyramidal side effects at clinically effective doses and possibly greater efficacy to reduce negative symptoms. As a group, they also have a superior effect on cognitive function and greater ability to treat mood symptoms in both patients with schizophrenia or affective disorders than typical antipsychotic drugs. The atypical antipsychotic agents vary in their affinities for other types of 5-HT as well as dopamine, muscarinic, adrenergic, and histaminic receptors, some, or all of which, may contribute to their differences in efficacy and side effect profile. Of the other 5-HT receptors which these drugs directly, the 5-HT_{1a} and 5-HT_{2c} receptors are the strongest candidates for contributing to their antipsychotic action and low EPS profile. The 5-HT_6 and 5-HT_7 receptors may also be of some importance. Stimulation of the 5-HT_{1a} receptor appears to produce many of the same effects as antagonism of the 5-HT_{2a} receptor while antagonism of the 5-HT_{2c} receptor appears to diminish some of the actions of 5-HT_{2a} receptor antagonism. Future antipsychotic drug development can include targeting multiple serotonin receptor subtypes.
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Recent interest in the role of serotonin (5-HT) in the mechanism of action of antipsychotic drugs is in large part the result of the discovery of the efficacy of clozapine in treating the delusions, hallucinations, and disorganization of schizophrenic patients who failed to respond to classical neuroleptic drugs (Kane et al. 1988; Meltzer 1992). It has also been demonstrated that clozapine can improve the negative symptoms of schizophrenia, i.e., affective flattening, anergia, anhedonia, and avolition (Meltzer et al. 1996). Clozapine was, earlier, shown to cause fewer extrapyramidal side effects (EPS) than typical antipsychotic drugs; there have been no reported cases of tardive dyskinesia with clozapine, and it is tolerable to patients with Parkinson's disease and even able to improve some types of motor dysfunction in patients who develop dopamine (DA) agonist-induced psychosis (see Meltzer and Nash 1991; Meltzer et al. 1995 for refs). Clozapine has also been found not to cause elevations of serum prolactin levels (Meltzer 1979). Recently, clozapine has been shown to have other clinical advantages over typical neuroleptic drugs, most notably the ability to improve some aspects of the cognitive dysfunction of schizophrenia, such as attention, verbal fluency (semantic memory), recall memory, and some measures of executive function, e.g., maze and performance, thought it does not improve working memory (Meltzer and McGurk in press). There is extensive evidence that clozapine is able to decrease the risk of suicide in schizophrenia (Meltzer and Okayli 1995). It has also been found effective as an antimanic agent and

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a mood stabilizer in treatment-resistant mood disorders (Calabrese et al. 1996). Suicide, mania, depression, and mood stabilization have been related to abnormalities in serotonergic availability and receptor responsivity (Maes and Meltzer 1995). Determining the biological basis for these advantages of clozapine, many of which may involve effects on 5-HT, is of great theoretical and clinical importance.

Because clozapine produces agranulocytosis, developing other antipsychotic drugs with similar benefits but without this side effect has been a major research goal since 1988. This effort has produced risperidone, olanzapine, quetiapine, ziprasidone, and sertindole which have been approved for use in the treatment of schizophrenia in various countries as well as other putative antipsychotic agents in development, e.g., M100907. However, there appear to be differences between these agents in efficacy and in at least some side effects. This review considers what is known about the role of 5-HT in the efficacy for positive and negative symptoms, neuroleptic refractory positive symptoms, and extrapyramidal side effects of the novel antipsychotic agents, and looks at possible strategies for developing other antipsychotic agents which depend upon serotonergic function in ways that differ from clozapine. Because of space limitation, this review cannot cover all of the topics in depth; therefore, other reviews of these topics also should be consulted (Meltzer and Nash 1991; Schmidt et al. 1995; Kapur and Remington 1996; Meltzer and Fatemi 1996; Abi-Dargham et al. 1997; Kinon and Lieberman 1996; Arndt and Skarsfeldt 1998).

SEROTONIN RECEPTORS INVOLVED IN ANTIPSYCHOTIC DRUG ACTION

The major 5-HT receptors that are implicated in the action of clozapine and other recently introduced antipsychotic agents, or are of potential value for developing more effective or better tolerated antipsychotic agents include the following: 5-HT_{1a}, 5-HT_{2a}, 5-HT_{2c}, 5-HT_3, 5-HT_6, and 5-HT_7 receptors (Meltzer and Nash 1991). These are also of potential value for developing more effective or better tolerated antipsychotic agents. It has been hypothesized that a relatively high 5-HT_{2a} receptor affinity compared to the D_2 receptor is the basis for the difference between 'atypical' and 'typical' antipsychotic agents (with atypical antipsychotic defined as an agent causing low EPS at doses with demonstrated or putative antipsychotic activity). This hypothesis contributed to the development of the newer antipsychotic agents listed above, all of which are consistent with the hypothesis of high affinity for 5-HT_{2a} and low affinity for D_2 receptors (Schotte et al. 1996). While some of the atypical antipsychotic drugs also have affinities for 5-HT_{2c}, 5-HT_6, or 5-HT_7 receptors that are in the same range as 5-HT_{2a} receptors, this is not a common characteristic of these agents and, thus, it is not likely that an affinity for 5-HT_{2c}, 5-HT_6, or 5-HT_7 receptors are primary factors contributing to the low EPS profile of this class of agents (Roth et al. 1994; Meltzer and Fatemi 1996; Schotte et al. 1996). However, this does not rule out the possibility that the low EPS effects of specific drugs or other actions, depend in part on their affinity for one or more of these 5-HT receptors, including the 5-HT_{2c} and 5-HT_7 receptors (Meltzer et al. 1996). 5-HT_{1a} receptor agonism has been suggested to contribute to an atypical antipsychotic drug profile (Protais et al. 1994). Furthermore, there is extensive evidence of interactions among 5-HT_{2a}, 5-HT_{2c}, and 5-HT_{1a} receptors (Kapur and Remington 1996; Meltzer et al. 1996b). Because of space limitations, this article will focus on these three 5-HT receptors and only briefly consider the others.

NOVEL ANTIPSYCHOTICS AND THE 5-HT_{2A} RECEPTOR ANTIPSYCHOTIC ACTION

5-HT_{2a} receptors have been implicated in the genesis of, as well as the treatment of, psychosis, negative symptoms, mood disturbance, and EPS. The hallucinogenic effect of indole hallucinogens has been related to stimulation of 5-HT_{2a} rather than 5-HT_{2c} receptors (Fiorella et al. 1995). Numerous studies have demonstrated decreased densities of 5-HT_{2a} receptors in various cortical regions of patients with schizophrenia. This does not appear to be secondary to antipsychotic drug-induced down regulation of the density of these receptors (Meltzer et al. 1996b). The antipsychotic effect of clozapine is attributed, in part, to its ability to block excessive 5-HT_{2a} receptor stimulation (Meltzer et al. 1989). This conclusion is supported by the high occupancy of 5-HT_{2a} receptors produced by clozapine at clinically effective doses and its low occupancy of D_2 receptors (in the 30–50% range as measured with the [^{3}H]raclopride significantly below the 80 to 100% occupancy produced by typical neuroleptic drugs) (Kapur and Remington 1996). The occupancy of 5-HT_{2a} and D_2 receptors has also been studied with other novel antipsychotic drugs, such as risperidone, olanzapine, sertindole, and quetiapine with results similar to those of clozapine; all are more potent 5-HT_{2a} and D_2 antagonists at appropriate doses, but produce higher D_2 occupancy than clozapine. Some of these (e.g., risperidone) agents, produce high D_2 occupancy at high doses (Kapur and Remington 1996). Pilowsky et al. (1997a) have observed high occupancy of extrastriatal D2 receptors in clozapine treated patients with schizophrenia in a SPECT study. These findings, if verified, could indicate that the low estimates of D_2 receptor occupancy in the basal ganglia by some atypical antipsychotic drugs in some studies are mis-

leading with regard to the importance of dopamine receptor blockade for their antipsychotic, and perhaps other actions, e.g., cognition and negative symptoms.

To test the contribution of 5-HT_{2a} receptor antagonism to antipsychotic drug action, clinical trials of ritanserin, a potent 5-HT_{2a} and 5-HT_{2c} antagonist, have been conducted. Although the data suggest little or no beneficial effect (see Martin et al. 1997 for refs), this may be due to two factors: a) 5-HT_{2c} receptor antagonism opposes the beneficial effects of 5-HT_{2a} receptor blockade (see below); and b) too high a dose of neuroleptic (Meltzer and Fatemi 1996). The bell-shaped dose response curve of risperidone, with higher doses being less effective than lower doses (Marder and Meibach 1994), suggests that excessive D_2 receptor antagonism may reduce the beneficial effects of 5-HT_{2a} receptor blockade (Meltzer and Fatemi 1996). However, other explanations of this finding are also possible. Trials with fixed, low doses of neuroleptic and specific potent 5-HT_{2a} antagonists are needed. Amperozide is a relatively selective 5-HT_{2a} antagonist which was shown in several studies to have antipsychotic efficacy but again no definite conclusion as to efficacy is possible (Meltzer and Nash 1991). The highly selective 5-HT_{2a} antagonist, M100907, formerly MDL 100907, has been found in a controlled study to have some efficacy for treating positive and negative symptoms in hospitalized schizophrenic patients (Shipley 1998).

Large scale studies are currently underway to more definitively test its efficacy. I hypothesize that this compound will be effective in some patients with schizophrenia, but that there will be a need for low doses of D_2 receptor antagonists in patients who may have relatively excessive dopaminergic activity. This may be those patients with acute, florid psychoses. Other 5-HT_{2a} selective agents such as SR 46349B (Rinaldi-Carmona et al. 1992) are currently being tested. It should soon be apparent whether 5-HT_{2a} receptor blockade alone can achieve a sustained antipsychotic action. The potent D_4/5-HT_{2a} receptor antagonist fananserin has been found ineffective in acutely psychotic patients with schizophrenia (Truffinet et al. in press), perhaps because D_4 receptor antagonism has a propsychotic effect, not the reverse. Additional evidence supporting the role of 5-HT_{2a} receptor blockade in the action of clozapine and possibly other drugs with potent 5-HT_{2a} affinities is available from the several reports that the *His452Tyr allele* of the 5-HT_{2a} receptor, which is present in 10–12% of the population, is associated with a higher frequency of poor response to clozapine (Masellis et al. 1998). Thus, taken together, the evidence from clinical trial data suggests that 5-HT_{2a} receptor blockade may contribute to antipsychotic drug action.

Basic research is also supportive of the relevance of 5-HT_{2a} receptor blockade to antipsychotic drug action. M100907 or other selective 5-HT_{2a} receptor antagonists, either alone or in combination with selective antagonists of other receptors, have been found to be effective in various animal models of psychosis. These include: 1) blockade of amphetamine-induced locomotor activity and the slowing of ventral tegmental area (A10) dopaminergic neurons (Schmidt et al. 1995); 2) blockade of phencyclidine (PCP)- and dizocilpin (MK-801)-induced locomotor activity (Gleason and Shannon 1997; Martin et al. 1997); 3) blockade of MK-801-induced prepulse inhibition (Varty and Higgins 1995); and 4) antipsychotic- like activity in the paw test.

Increased dopaminergic activity in the nucleus accumbens, other mesolimbic and possibly cortical regions may contribute to positive symptoms, including formal thought disorder. We have previously reported that the 5-$HT_{2a/2c}$ agonist DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) which itself had no effect on basal DA release, potentiated amphetamine-induced DA release and attenuated the ability of apomorphine, a direct acting $D_{1/2/3}$ agonist, to decrease DA release in the striatum (Ichikawa and Meltzer 1995). We have proposed that short-lasting desensitization of D_2 autoreceptors can develop following stimulation of 5-HT_{2a} receptors. This desensitization may increase DA synthesis via disinhibition of tyrosine hydroxylase, leading to an additional increase in the size of the DA pool at which amphetamine may act. We have found a similar effect in the nucleus accumbens and prefrontal cortex (Ichikawa and Meltzer 1995). This effect was completely reversed by M100907, which alone had no effect on basal and amphetamine-induced DA release in the nucleus accumbens and medial prefrontal cortex. These results suggest that stimulated DA release, e.g., with stress, may be increased in the forebrain terminal regions secondary to enhanced stimulation of 5-HT_{2a} receptors. Agents that block the effect of excessive, but not basal 5-HT_{2a} receptor stimulation may be most useful clinically. Schmidt et al. (1995) reported that M100907 increased DA release in the prefrontal cortex and attenuate the amphetamine analog MDMA – induced increase in striatal DA release. Taken together, these data suggest that 5-HT_{2a} antagonism by itself may have antipsychotic action when dopaminergic activity is slightly to moderately increased, but as mentioned above some D_2 receptor blockade may also be needed when DA efflux is very high. More studies are needed to define the ability of 5-HT_{2a} receptor antagonists to potentiate the action of low doses of D_2 receptor blockers in animal models as well as in humans.

Recently, Jakab and Goldman-Rakic (1998) have proposed that the 5-HT_{2a} receptors on cortical pyramidal neurons may play a crucial role in psychosis by virtue of their ability to modulate intracortical and cortical-subcortical glutamatergic neurotransmission. This could contribute to the ability of 5-HT_{2a} antagonists to attenuate some of the behavioral effects of PCP and ketamine.

On the other hand, activation of early intermediate genes such as c-*fos* in the frontal cortex by clozapine and other novel antipsychotic agents (Robertson et al. 1994), does not appear to be related to blockade of 5-HT_{2a} receptors since ritanserin alone, or the combination of ritanserin and sulpiride, a D_2/D_3 antagonist, did not mimic the effects of clozapine to activate Fos expression in the prefrontal cortex (Deutch and Duman 1996). As in many studies which employ this combination strategy, the dose of the D_2/D_3 antagonist may have been excessive. It would be of considerable interest to study the effect of a series of doses of M100907, a selective 5-HT2a receptor antagonist, and relatively low doses of a selective D_2/D_3 antagonist, on rat brain c-*fos* expression. On the other hand, lesioning the serotonergic afferents to the medial prefrontal cortex also did not affect the ability of clozapine to activate Fos protein in the frontal cortex (Guo et al. 1995). It is important to note that the 5-$HT_{2a/2c}$ agonist (DOI), increased the expression of fos protein in frontal and other cortical and limbic areas (Leslie et al. 1993), most likely through a 5-HT_{2a} mechanism. Thus, it seems unlikely that 5-HT_{2a} receptor antagonism is involved in the ability of the atypical antipsychotic drugs to stimulate fos expression in the prefrontal cortex. Recent studies have suggested that D_3 receptor stimulation may account for this effect (Guo et al. 1995). While the activation of c-*fos* in the frontal cortex seems not to be related to 5-HT_{2a} receptor antagonism, it nevertheless seems likely that the latter contributes to the antipsychotic action of the atypical antipsychotic drugs when associated with low D_2 receptor occupancy, and not accompanied by effects such as 5-HT_{2c} or possibly D_4 receptor antagonism which may negate some of the beneficial effects of 5-HT_{2a} receptor antagonism. It should be kept in mind that the clinical significance of activation of c-fos expression in the frontal cortex is still quite speculative.

TREATMENT RESISTANT SCHIZOPHRENIA AND 5-HT_{2A} RECEPTOR BLOCKADE

Clinical studies have suggested that clozapine is much more effective than the other novel antipsychotic drugs in treatment resistant schizophrenia (Meltzer 1997). One recent study found no effect of high doses of olanzapine in treatment resistant schizophrenia (Conley et al. 1998a), though there is limited published evidence that risperidone is effective in this patient group (Bondolfi et al. 1998). However, there is extensive clinical experience that some patients who are refractory to typical neuroleptic drugs, and even clozapine, may respond to olanzapine or risperidone (Conley et al. 1998b; Ganguli and Brar 1998; Mountjoy et al. 1998). Head to head comparisons with clozapine and cross over studies are needed to determine the relative efficacy of the atypical agents in this regard. It should be noted that there are varying degrees of neuroleptic resistance and that studies which are designed to determine whether other antipsychotic drugs are as effective as clozapine for treatment resistant schizophrenia should use rigorous criteria for neuroleptic resistance. The relatively modest effects of drugs such as olanzapine, quetiapine and risperidone in treatment resistant schizophrenia suggests that 5-HT_{2a} receptor blockade, even when accompanied by D_2 receptor blockade, is only one facet of the explanation of the efficacy of clozapine in neuroleptic-resistant schizophrenic patients. Effects on other 5-HT receptors as well as cholinergic, adrenergic, and dopaminergic mechanisms may be responsible for the greater efficacy of clozapine in this group of patients. Heterogeneity in the basis for neuroleptic resistance is suggested by the variability in response to the atypical antipsychotic drugs. We reported preliminary data that the addition of cyproheptadine, an antagonist of multiple serotonin receptors, to typical neuroleptic drugs appeared to be effective in augmenting the response to the latter in an open study of patients who had become refractory to typical neuroleptic drugs during the course of treatment with clozapine (Meltzer et al. 1996a).

NEGATIVE SYMPTOMS AND 5-HT_{2A} RECEPTOR BLOCKADE

In contrast to the possibly unique effectiveness of clozapine in diminishing positive symptoms in treatment resistant schizophrenic patients, the atypical antipsychotic drugs (with the possible exception of quetiapine) and some 5-HT_{2a} or 5-$HT_{2a/2c}$ antagonists, appear to be more effective in decreasing negative symptoms than haloperidol, (Kane et al. 1988; Meltzer 1992, 1997; Moller et al. 1995; Tollefson and Sanger 1996). Sertindole, risperidone, ziprasidone, M100907, and ritanserin have all been found effective in treating negative symptoms (Arvanitis and Miller 1997; Arato et al. submitted). Various types of analyses to partial out the effect on negative symptoms from the effect on positive and depressive symptoms and EPS suggest that the effect on negative symptoms is direct (Moller et al. 1995; Tollefson and Sanger 1996). These diverse compounds share relatively high 5-HT_{2a} receptor blockade without D_2 antagonism, whereas chlorpromazine and high dose loxapine, which produce high levels of D_2 and 5-HT_{2a} receptor blockade in vivo, do not improve negative symptoms. This suggests that 5-HT_{2a} receptor blockade may play a key role in the treatment of negative symptoms only when D_2 receptor blockade is absent or moderate. Efficacy to treat negative symptoms may be related to the ability of these agents to selectively increase dopaminergic activity in the prefrontal cortex since all these agents have been found to produce greater in-

creases in DA release in the prefrontal cortex than the nucleus accumbens (Kuroki et al. 1999).

5-HT_{2A} RECEPTOR BLOCKADE AND COGNITIVE FUNCTION

Clozapine, risperidone, and olanzapine have all been shown to improve selected areas of cognitive function in patients with schizophrenia, however, the available data suggests differential effects on specific functions. Most notably, clozapine appears to improve semantic memory, verbal learning and memory, and attention most reliably and robustly, whereas risperidone appears to improve working memory to the greatest extent (see Meltzer and McGurk in press for review). Olanzapine resembles clozapine more closely than risperidone (Purdon et al. 1998; Meltzer and McGurk in press). For example, in a study of 21 olanzapine treated patients, Purdon et al. (1998) reported improvement in some tests of attention, motor function, executive function and verbal memory at six weeks. Similar results were obtained by Meltzer and McGurk (in press) in a study of 29 patients treated with olanzapine for six weeks. Whether these effects are persistent and independent of improvement in psychopathology or lesser side effects is not yet known. There are, as yet, no data on the other drugs of this type. Whether 5-HT_{2a} receptor antagonism has any role in the cognitive effects of these agents is not known. It may be that the effects of these agents on cognition depend mainly on their ability to increase the release of DA and acetylcholine in prefrontal cortex, which may depend, in part, on their serotonergic actions. Because cognitive enhancement is critical for functional improvement in schizophrenia, establishing the mechanism for this effect is of the greatest importance. The evidence concerning 5-HT receptors and cognition has recently been reviewed by Buhot (1997) and by Meneses and Hong (1997). The sparse literature on this topic mainly involves lower animals; the data suggest that 5-$HT_{2a/2c}$ antagonists have little adverse effects and no apparent beneficial effects on learning and memory (Ruotsalainen et al. 1997).

Recent studies suggest that the novel antipsychotic drugs may enhance cholinergic function in the prefrontal cortex (Parada et al. 1997), and this may mediate their ability to improve cognition. Interactions between the 5-HT and cholinergic systems have been previously reported (Altman et al. 1987). Also, impairment of working memory in man following administration of the 5-HT_{1a} agonist, flesinoxan, has been reported (Herremans et al. 1995).

It has recently been demonstrated that stimulation of 5-HT_{2a} receptors by DOI increases the expression of brain-derived neurotrophic factor (BDNF), which regulates the survival, differentiation, synaptic strength, and neuronal morphology in the cerebral cortex and hippocampus, in frontal and other cortical areas while decreasing its expression in the dentate gyrus granule cell layer (Vaidya et al. 1997). This effect was blocked by M100907 which did not by itself have any effect in these regions. Stress had the same effect as DOI in increasing hippocampal BDNF levels. This was blocked by pretreatment with ketanserin, a 5-HT_{2a} antagonist. These data suggest that the atypical antipsychotic drugs, via their 5-HT_{2a} antagonism, might prevent the deleterious effects of stress on hippocampal cognitive measures. Increased corticosteroids may be neurotoxic (Sapolsky 1994). We have previously shown that clozapine decreases elevated plasma cortisol levels in schizophrenic patients, returning them to normal levels (Meltzer et al. 1989). This may be related to the ability of clozapine to improve cognitive function as well.

5-HT_{2A} RECEPTOR BLOCKADE AND EXTRAPYRAMIDAL FUNCTION

Several lines of evidence suggest that potent 5-HT_{2a} receptor blockade is related to the low EPS profile of clozapine but that 5-HT_{2a} receptor blockade by itself cannot explain the low EPS liability of these agents. Altar et al. (1986) and Rasmussen and Aghajanian (1988) suggested that weak D_2 and potent 5-HT_{2a} receptor blockade was the basis for the ability of clozapine to cause low extrapyramidal side effects. However, other explanations for the low EPS of clozapine have been offered—namely its anticholinergic properties, lack of ability to increase acetylcholine in the striatum, D_1 and D_4 receptor blockade, and its effects as an α_2-adrenoceptor antagonist (Meltzer and Fatemi 1996; Parada et al. 1997; Kalkman et al. 1998).

In order to test the role of 5-HT_{2a} receptor blockade, Meltzer et al. (1989) studied a group of compounds which had antipsychotic activity in man or in animal models and which produced less EPS in man or weak catalepsy in animals. Among the drugs studied was melperone, a butyrophenone long used in Europe and Scandinavia as an antipsychotic and reported to produce low EPS (Meltzer et al. 1994). This drug has recently been found to be tolerable to patients with Parkinson's Disease (Barbato et al. 1996), even more so than risperidone andolanzapine. The drugs reportedly shared weak D_2 and potent 5-HT_{2a} receptor affinities, whereas D_1 receptor affinities did not contribute to their effect. Subsequently, numerous compounds of diverse chemical structure which share this pharmacologic profile have been deliberately synthesized and tested for antipsychotic action and EPS potential. They include risperidone, olanzapine, sertindole, quetiapine, ziprasidone, and olanzapine. In development are iloperidone, M100907, and SR43649B; the latter is virtually devoid of

any D_2 affinity, and highly selective for the 5-HT_{2a} receptors. All of these compounds produce fewer EPS than haloperidol at comparable doses, but there are differences between them. The dose response curve, together with PET studies of DA receptor occupancy strongly suggest that keeping 5-HT_{2a} receptor high relative to D_2 receptor occupancy is necessary to avoid EPS with these compounds. There are preclinical data to support this. Ishikane et al. (1997) reported that M 100907 is able to block haloperidol-induced catalepsy only at low doses of haloperidol. Similarly, Spampinato et al. (1998) reported that specific 5-HT_{2a} and 5-HT_{2c} antagonists were able to modulate the ability of haloperidol 0.01 mg/kg, but not 1.0 mg/kg to increase striatal DA release in freely moving rats. It should be noted that Pilowsky et al. (1997b) found high D_2 receptor occupancy in the basal ganglia in sertindole-treated patients, despite low EPS. This would indicate that some feature of the drugs, perhaps high 5-HT_{2a} receptor occupancy, but conceivably other aspects of its pharmacology as well, might contribute to the low EPS and overcome the effects of a high degree of D_2 receptor blockade. It should be noted that the relative affinities for 5-HT_{2a} and D_2 receptors does not appear to be relevant to the ability of antipsychotics to induce dystonias in Cebus monkeys (Casey 1993). However, the relevance of these data to human EPS may be limited. For example, as noted above, melperone is well tolerated in patients with Parkinson's disease and L-DOPA psychosis who are exquisitely sensitive to drugs which cause EPS (Meltzer et al. 1989) but it produces dystonia in monkeys (Casey 1991).

THE ROLE OF THE 5-HT_{2C} RECEPTOR IN ANTIPSYCHOTIC DRUG ACTION: 5-HT_{2A} AND 5-HT_{2C} INTERACTIONS

No significant differences have been reported between novel antipsychotic drugs and typical neuroleptics with regard to the affinity for 5-HT_{2c} receptor or the difference between 5-HT_{2c} and D_2 affinities (Roth et al. 1992, 1994; Schotte et al. 1996). Of the approved novel antipsychotic drugs, some have equivalent affinities for the 5-HT_{2a} and 5-HT_{2c} receptors (clozapine, olanzapine, sertindole), while others are more selective for the 5-HT_{2a} receptor (risperidone, quetiapine, ziprasidone). This difference roughly corresponds to the potential to produce weight gain in that clozapine and olanzapine cause the greatest weight gain, and risperidone and ziprasidone the least. There is little available data for sertindole and quetiapine but they appear to be intermediate.

There is no apparent relationship between 5-HT_{2c} affinity and 5-HT_{2a} affinity with regard to EPS, since quetiapine and ziprasidone are comparable to olanzapine and sertindole in this regard. Similarly, there is no apparent relationship to efficacy in treatment-resistant schizophrenia. There could be a relationship to cognitive function since olanzapine and clozapine are similar to each other and differ from risperidone, but further studies are needed to determine the cognitive effects of all these agents before firm conclusions can be drawn (Meltzer and McGurk in press). 5-HT_{2c} receptor stimulation by the selective 5-HT_{2c} agonist Ro 60-0175 has been reported to markedly suppress dialysate levels of dDAn and noradrenaline in the frontal cortex of awake freely moving rats whereas the selective 5-HT_{2c} antagonist, SB-242084, had the opposite effect (Millan et al. 1998). Thus, the 5-HT_{2c} antagonist effects of some of the atypical antipsychotic drugs may contribute to their ability to increase dopaminergic activity in the prefrontal cortex.

An interesting aspect of the 5-HT_{2c} receptor with regard to antipsychotic action is that 5-HT_{2c} antagonism may be functionally opposed to 5-HT_{2a} antagonism. Meltzer et al. (1996) reported that atypical antipsychotic drugs were more likely to be weak 5-HT_{2c} and potent 5-HT_{2a} antagonists than to typical neuroleptic drugs. Neurochemical (Spampinato et al. 1997, 1998) and behavioral (Martin et al. 1997) data have now been reported supporting the notion of a functional antagonism of these two receptors which may coexist on the same neurons. Thus, Martin et al. (1997) found that ritanserin, a mixed 5-$HT_{2a/2c}$ antagonist, blocked the ability of M100907 to antagonize the effect of MK-801 in increasing locomotor activity in mice.

THE ROLE OF THE 5-HT_{1A} RECEPTOR IN ANTIPSYCHOTIC DRUG ACTION: 5-HT_{1A} AND 5-HT_{2A} INTERACTIONS

Although there is limited clinical data to support the role of the 5-HT_{1a} receptor in the action of antipsychotic drugs, there is increasing amounts of preclinical evidence that it is of considerable importance. The 5-HT_{1a} receptor is located pre- and postsynaptically. The presynaptic 5-HT_{1a} receptor is an autoreceptor located on cell bodies of raphe neurons; stimulation leads to inhibition of the firing of 5-HT neurons. Stimulation of postsynaptic 5-HT_{1a} receptors leads to hyperpolarization of neurons – the opposite effect of stimulation of 5-HT_{2a} receptors.

Extensive evidence indicates that 5-HT_{1a} receptor agonists have effects similar to 5-HT_{2a} receptor antagonists in a variety of systems (Darmani et al. 1990; Meltzer and Maes 1995). A few examples will be given. DOI injected bilaterally into the rat medial prefrontal cortex elicited a dose-dependent head twitch response. This effect wa inhibited by M100907 and ketanserin, but the selective 5-HT_{2c} antagonist SDZ SER082. Pretreatment with the 5-HT_{1a} agonist 8-OH-DPAT inhibited the head

twitch response to DOI. Ahlenius (1988) first suggested that stimulation of 5-HT_{1a} receptors might produce an antipsychotic-like effect, based on behavioral studies in animals using the direct 5-HT_{1a} agonist 8-OH-DPAT.

Subsequent studies demonstrated that 8-OH-DPAT enhanced the antipsychotic-like effect of the D_2/D_3 antagonist raclopride (Wadenberg and Ahlenius 1991) and of haloperidol (Prinssen et al. 1996), and antagonized the catalepsy induced by the D_1 agonist SCH23390 in rats (Wadenberg 1992). The beneficial effect of 5-HT_{1a} agonists appears to be mediated by inhibition of median raphe serotonergic neurons (Wadenberg and Hillegaart 1995). Ichikawa and Meltzer (1995) demonstrated that 8-OH-DPAT inhibited the ability of clozapine and low dose risperidone but not haloperidol to increase extracellular DA levels in the nucleus accumbens and the striatum of conscious rats. The effect in the nucleus accumbens would be expected to enhance the antipsychotic effect of these agents by reducing dopaminergic activity. Several atypical antipsychotic drugs, including clozapine, ziprasidone, quetiapine, and tiospirone, are partial agonists at the 5-HT_{1a} receptor. Their affinities for the 5-HT_{1a} receptor were similar to their affinities at the human D_2 dopamine receptor (Newman-Tancredi et al. 1998). Rollema et al (1997) demonstrated that the ability of clozapine to increase DA release in the rat prefrontal cortex was due to its 5-HT_{1a} agonist properties, in that it could be blocked by WAY-100635, a 5-HT_{1a} antagonist. These findings suggest that the combination of D_2 antagonism and 5-HT_{1a} agonism should produce an atypical antipsychotic agent. S16924 is an example of such a compound and it has atypical properties very similar to those of clozapine in a variety of animal models (Millan et al. 1998a, 1998b). How closely such compounds will parallel 5-HT_{1a} /D_2 antagonists remains to be determined. Significant differences should be expected. Compounds such as ziprasidone and clozapine combine 5-HT_{1a} agonism and 5-HT_{2a} antagonism.

Clinical studies of adding 5-HT_{1a} partial agonists such as buspirone, ipsapirone, and tandospirone may help to clarify the possible importance of 5-HT_{1a} agonism in the treatment of schizophrenia. It seems likely that there could be a new generation of antipsychotic drugs that incorporate 5-HT_{1a} agonism.

THE ROLE OF SEROTONIN RELEASE IN ANTIPSYCHOTIC DRUG ACTION

The antagonism of multiple 5-HT receptors by clozapine would be expected to enhance the release of 5-HT by feedback mechanisms. Thus, it was surprising that Ferré and Artigas (1995) reported that clozapine decreased 5-HT release in the nucleus accumbens. However, Ichikawa et al. (1998) reported that clozapine (20 mg/kg) and risperidone (1 mg/kg) significantly increased extracellular 5-HT levels in the nucleus accumbens and medial prefrontal cortex, respectively, whereas amperozide (1 and 10 mg/kg) increased extracellular 5-HT levels in both regions. Hertel et al. (1997) reported similar results with risperidone and suggested that they might be releated to its ability to improve negative symptoms. If so, this is not the explanation for the effects of clozapine or olanzapine on negative symptoms since olanzapine, sulpiride, haloperidol, and M100907 had no effect on extracellular 5-HT levels in either region. The latter finding indicates that blockade of 5-HT_{2a} receptors is not the basis for the ability of clozapine, risperidone or amperozide to increase 5-HT_{2a} levels (Ichikawa et al. 1998). The enhancement of 5-HT efflux in the prefrontal cortex may contribute to the ability of these agents to improve mood disorders and cognition.

CONCLUSIONS

There is strong evidence for the role of 5-HT_{2a} receptors and suggestive evidence for the roles of the 5-HT_{1a} and 5-HT_{2c} receptors in the antipsychotic effects and ability to improve cognition of arious actions of clozapine, risperidone, olanzapine, quetiapine, ziprasidone, iloperidone, sertindole, and related antipsychotic drugs. Studies of specific antagonists and agonists of these receptors, alone and together with D_2 receptor antagonists and dopaminomimetic agents such as L-DOPA, amphetamine, and others will help to clarify their importance. Thus, if selective 5-HT_{2a} antagonists such as M100907 and SR 46439B are effective in the treatment of auditory hallucinations or paranoid delusions induced by pharmacologically enhanced dopamine activity, the case for the importance of 5-HT_{2a} receptor blockade in the treatment of psychosis will be supported. There is considerable evidence that 5-HT_{2a} receptor antagonism is most usefully conceptualized in relation to dopamine receptor antagonism. D_2 receptors appears to be the most important in this regard but it is likely that the D_1, D_3, and D_4 receptors will also influence various components of psychosis, mood, motivation, and cognition that are relevant to the clinical applications of the atypical antipsychotic drugs.

Potent 5-HT_{1a} agonism, and possibly 5-HT_{2c} receptor agonism and 5-HT_6 or 5-HT_7 receptor antagonism may also be the basis for some of the actions of the current generation of atypical antipsychotic drugs and the basis for developing novel agents that do not necessarily have to be 5-HT_{2a} antagonists. Multi-receptor agents appear to be more promising as antipsychotic agents for the majority of psychiatric patients because of important interactions between neural circuits which employ multiple neurotransmitters. Underlying the efficacy of antipsychotic drugs which affect the serotonergic system is probably abnormalities in the serotonergic system

itself, e.g., increased 5-HT_{2a} and possibly decreased 5-HT_{1a} receptor stimulation. However, the case of Parkinson's psychosis induced by L-DOPA or bromocriptine suggests that drugs such as clozapine and melperone may be effective antipsychotics via their 5-HT_{2a} receptor antagonist properties even when the primary abnormality is mainly excessive dopaminergic stimulation.

The limitations in the scope of this review to some of the literature on serotonin and dopamine with regard to the action of the new antipsychotic drugs necessitated omitting attention to the importance of other neurotransmitters such as acetylcholine, glutamate and norepinephrine. These neurotransmitters most certainly contribute to the morbid state in psychosis and are influenced by the newer agents directly or indirectly. Despite the importance of these other neurotransmitters, only the antipsychotic drugs which have a spectrum of effects on the serotonergic system, particularly the 5-HT2a receptor, have produced highly relevant advances in the treatment of psychosis. There are no antipsychotic drugs yet available which have primary effects on any of these other neurotransmitters. This provides supports for a continued focus on serotonin, and especially serotonin-dopamine interactions, with regard to antipsychotic drug actions.

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REFERENCES

Abi-Dargham A, Laruelle M, Aghajanian GK (1997): The role of serotonin in the pathophysiology and treatment of schizophrenia. J Neuropsy Clin Neurosci 9:1–17

Ahlenius S (1988): Antipsychotic-like properties of the 5-HT_{1a} agonist 8-OH-DPAT in the rat. Pharmacol Toxicol 64:3–5

Altman HJ, Stone WS, Ögren S (1987): Evidence for a possible functional interaction between serotonergic and cholinergic mechanism in memory retrieval. Behav Neural Biol 48:49–62

Altar CA, Wasley AM, Neale RF, Stone GA (1986): Typical and atypical antipsychotic occupancy of D_2 and S_2 receptors: An autoradiographic analysis in rat brain. Brain Res Bull 16:517–525

Arndt J, Skarsfeldt T (1998): Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. Neuropsychopharmacology 18:63–101

Arvanitis LA, Miller BG (1997): Multiple fixed dose of "Seroquel" (quetiapine) in patients with acute exacerbation of schizophrenia: A comparison with haloperidol and placebo. The Seroquel Trial 13 Study Group. Biol Psychiatry 42:233–246

Barbato L, Monge A, Stocchi F, Nordera G (1996): Melperone in the treatment of iatrogenic psychosis in Parkinson's Disease. Clin Neuropharmacol 11:201–207

Bondolfi G, Dufour H, Patris M, May JP, Billeter U, Eap CB, Baumann P (1998): Risperidone versus clozapine in treatment resistant chronic schizophrenia: A randomized double-blind study. The Risperidone Study Group. Am J Psychiatry 155:499–504

Buhot M-C (1997): Serotonin receptors in cognitive behaviors. Curr Opin Neurobiol 7:243–254

Calabrese J, Kimmel SE, Woyshiville M, Rapport WJ, Trivedi CJ, Meltzer HY (1996): Clozapine in treatment refractory mania. Am J Psychiatry 153:759–764

Casey DE (1991): Extrapyramidal syndromes in nonhuman primates: Typical and atypical neuroleptics. Psychopharmacol Bull 27:47–50

Casey DE (1993): Serotonergic and dopaminergic aspects of neuroleptic-induced extrapyramidal syndromes in nonhuman primates. Psychopharmacology 112(Suppl 1): S55–S59

Conley RR, Tamminga CA, Bartko JJ, Richardson C, Peszke M, Lingle J, Hegerty J, Love R, Counaris C, Zaremba S (1998a): Olanzapine compared with chlorpromazine in treatment-resistant schizophrenia. Am J Psychiatry 155:914–920

Conley RR, Kelly DL, Gale EA (1998b): Olanzapine response in treatment-refractory schizophrenic patients with a history of substance abuse. Schiz Res 33:95–101

Darmani NA, Martin BR , Pandy U, Glennon RA (1990): Do functional relationships exist between 5-HT_{1a} and 5-HT_2 receptors? Pharmacol Biochem Behav 26:901–906

Deutch AY, Duman RS (1996): The effects of antipsychotic drugs on fos protein expression in the prefrontal cortex: Cellular localization and pharmacological characterization. Neuroscience 70:377–389

Ferré S, Artigas F (1995): Clozapine decreased serotonin extracellular levels in the nucleus accumbens by a dopamine receptor-independent mechanism. Neurosci Lett 187:61–64

Fiorella D, Helsley S, Rabin RA, Winter JC (1995): The interactions of typical and atypical antipsychotics with the (-)2,5-dimethoxy-4-methamphetamine (DOM) discriminative stimulus. Neuropharmacology 34:1297–303

Ganguli R, Brar JS (1998): The effects of risperidone and olanzapine on the indications for clozapine. Psychopharmacol Bull 34(1):83–87

Gleason SC, Shannon HE (1997): Blockade of phencyclidine-induced hyperlocomotion by olanzapine, clozapine and serotonin receptor subtype selective antagonists in mice. Psychopharmacology 129:79–84

Guo N, Klitenick MA, Tham CS, Fibiger HC (1995): Receptor mechanism mediating clozapine-induced *c-fos* expression in the forebrain. Neuroscience 65:747–756

Herremans AH, Hijzen TH, Olivier B, Slangen JL (1995): Serotonergic drug effects on a delayed conditional task in the rat: Involvement of the 5-HT_{1a} receptor in working memory. J Psychopharmacol 9:242–250

Hertel P, Nomikos G, Schilstrom B, Arborelius L, Svensson TH (1997): Risperidone dose-dependently increases extracellular concentrations of serotonin in the rat fron-

tal cortex: Role of α_2- adrenoceptor antagonism. Neuropsychopharmacology 17:44–55

Ichikawa J, Kuroki T, Dai J, Meltzer, HY (1998): Effect of antipsychotic drugs on extracellular serotonin levels in rat medial prefrontal cortex and nucleus accumbens. Eur J Pharmacol 351:163–171

Ichikawa J, Kuroki T, Kitchen MT, Meltzer HY (1995): R(+)-8-OH-DPAT, a 5-HT_{1a} receptor agonist, inhibits amphetamine-induced serotonin and dopamine release in rat striatum and nucleus accumbens. Eur J Pharmacol 287:179–184

Ichikawa J, Meltzer HY (1995): DOI, a 5-$HT_{2a/2c}$ receptor agonist, potentiates amphetamine-induced dopamine release in rat striatum. Brain Res 698:204–208

Ishikane T, Kusumi I, Matsubara R, Matsubara S, Koyama T (1997): Effects of serotonergic agents on the up-regulation of dopamine D2 receptors induced by haloperidol in rat striatum. Eur J Pharmacol 321:163–169

Jakab RL, Goldman-Rakic P (1998): 5-Hydroxytryptamine 2a serotonin receptors in the primate cerebral cortex: Possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. Proc Natl Acad Sci USA 95:735–740

Kalkman HO, Neumann V, Hoyer D, Tricklebank MD (1998): The role of a_2-adrenoceptor antagonism in the anti-cataleptic properties of the atypical neuroleptic agent, clozapine, in the rat. Br J Pharmacol 124:1550–1556

Kane J, Honigfeld G, Singer J, Meltzer HY and the Clozaril Collaborative Study Group (1988): Clozapine for the treatment-resistant schizophrenic. Arch Gen Psychiatry 45:789–796

Kapur S, Remington G (1996): Serotonin-dopamine interaction and its relevance to schizophrenia. Am J Psychiatry 153:466–476

Kinon BJ, Lieberman JA (1996): Mechanisms of action of atypical antipsychotic drugs—A critical analysis [review]. Psychopharmacology 124:2–34

Kuroki T, Meltzer HY, Ichikawa J (1999): Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. J Pharmacol Exp Ther 288:774–781

Leslie RA, Moorman JM, Coulson A, Grahame-Smith DG (1993): $Serotonin_{2/1C}$ receptor activation causes a localized expression of the immediate-early gene c-fos in rat brain: Evidence for involvement of dorsal raphe nucleus projection fibres. Neuroscience 53:457–463

Maes M, Meltzer HY (1995): Chapter 81. The serotonin hypothesis of depression. In Nemeroff CB, Kupfer D (eds), Psychopharmacology: The Fourth Generation of Progress. New York, NY, Raven Press, pp 933–944

Marder SR, Meibach RC (1994): Risperidone in the treatment of schizophrenia. Am J Psychiatry 151:825–835

Martin P, Water N, Carlsson A, Carlsson ML (1997): The apparent antipsychotic action of the 5-HT_{2a} receptor antagonist M100907 in a mouse model of schizophrenia is counteracted by ritanserin. J Neural Transm 104:561–564

Masellis FM, Macciardi FM, Meltzer HY, Lieberman JA, Sevy S, Cavazzoni P, Cola P, Howard A, Badri F, Nothen M, Kalow W, Kennedy JL (1998): Serotonin subtype 2 receptor genese and clinical response to clozapine in schizophrenic patients. Neuropsychopharmacology 19:123–132

Meltzer HY (1979): The clozapine story. In Hertzman M, Feltner DE (eds), The Handbook of Psychopharmacology Trials. New York, NY, New York University Press, pp 137–156

Meltzer HY (1992): Treatment of the neuroleptic non-responsive schizophrenic patient. Schiz Bull 18:515–542

Meltzer HY (1997): Treatment-resistant schizophrenia—The role of clozapine. Curr Med Res Op 14:1–20

Meltzer HY, Fatemi SH (1996): The role of serotonin in schizophrenia and the mechanism of action on anti-psychotic drugs. In Kane JM, Moller HJ, Awouters F (eds), Serotonergic Mechanisms in Antipsychotic Treatment. New York, NY, Marcel Decker, pp 77–107

Meltzer HY, Kennedy J, Dai J, Parsa M, Riley D (1995): Plasma clozapine levels and the treatment of L-DOPA-induced psychosis in Parkinson's disease: A high potency effect of clozapine. Neuropsychopharmacology 12:39–45

Meltzer HY, Lee MA, Ranjan R, Mason ED, Cola PA (1996a): Relapse following clozapine withdrawal: Effect of cyproheptadine plus neuroleptic. Psychopharmacology 124:176–187

Meltzer HY, Maes M (1995): Pindolol pretreatment blocks stimulation by metachlorophenylpiperazine of prolactin but not cortisol secretion in normal men. Psychiatry Res 58:89–98

Meltzer HY, Matsubara S, Lee J-C (1989): Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin2 pKi values. J Pharmacol Exp Ther 251:238–246

Meltzer HY, McGurk SR (in press): The effect of clozapine, risperidone and olanzapine on cognitive function in schizophrenia. Schiz Bull

Meltzer HY, Nash JF (1991): Effects of antipsychotic drugs on serotonin receptors. Pharmacol Rev 43:587–604

Meltzer HY, Okayli G (1995): The reduction of suicidality during clozapine treatment in neuroleptic-resistant schizophrenia: Impact on risk-benefit assessment. Am J Psychiatry 152:183–190

Meltzer HY, Roth B, Thompson P (1996b): Serotonin and dopamine receptor affinites predict atypical antipsychotic drug (AAD) activity. Neurosci Abstr 22:480

Meneses A, Hong E (1997): A pharmacological analysis of serotonergic receptors: Effect of their activation of blockade in learning. Prog Neuropsychopharmacol Biol Psychiatry 21:273–296

Millan MJ, Dekeyne A, Gobert A (1998a): Serotonin $(5\text{-}HT)_{2c}$ receptors inhibit dopamine (DA) and noradrenaline (NA), but not 5-HT, release in the frontal cortex in vivo. Neuropharmacology 27:953–955

Millan MJ, Gobert A, Newman-Tancredi A, Audinot V, Lejeune F, Rivet J-M, Cussac D, Nicolas J-P, Muller O, Lavielle G (1998b): S16924 ((R)-2-{1-[2-(2,3-dihydrobenzo[1,4] dioxin-5-yloxy)-ethyl]-pyrrolidin-3yl}-1-(4-fluoro-phenyl)-ethanone), a novel potential antipsychotic with marked serotonin 5-HT_{1A} agonist properties. 1. Receptorial and neurochemical profile in comparison with clozapine and haloperidol. J Pharmacol Exp Ther 286:1341–1355

Moller HJ, Muller H, Borison RL, Schooler NR, Chouinard G (1995): A path-analytical approach to differentiate between direct and indirect drug effects on negative symptoms in schizophrenic patients. A re-evaluation of the North American risperidone study. Euro Arch Psychiatry Clin Neurol 245:45–49

Mountjoy CQ, Baldacchino AM, Stubbs JH (1998): British experience with high-dose olanzapine for treatment-refractory schizophrenia. Am J Psychiatry 156:158–159

Newman-Tancredi A, Gavaudan S, Conte C, Chaput C, Touzard M, Verrièle L, Audinot V, Millan MJ (1998): Agonist and antagonist actions of antipsychotic agents at 5-HT_{1a} receptors: A [^{35} S]GTPγ S. Eur J Pharmacol 355:245–256

Parada MA, Hernandez L, Puig de Parada M, Rada P, Murzi E (1997): Selection action of acute systemic clozapine on acetylcholin release in the rat prefrontal cortex by reference to the nucleus accumbens and striatum. J Pharmacol Exp Ther 281:582–588

Pilowsky LS, Mulligan RS, Acton PD, Ell PJ, Costa DC, Kerwin RW (1997a): Limbic selectivity of clozapine. Lancet 350:490–491

Pilowsky LS, O'Connell PO, Davies N, Busatto GH, Costa DA, Murray RM, Ell PJ, Kerwin RW (1997b): In vivo effects on striatal dopamine D_2 receptor binding by the novel atypical antipsychotic drug sertindole—A ^{123}I IBZM single photon emission tomography (SPET) study. Psychopharmacology 130:152–158

Prinssen EPM, Kleven MS, Koek W (1996): Effects of dopamine antagonists in a two-way active avoidance procedure in rats: Interactions with 8-OH-DPAT, ritanserin, and prazosin. Psychopharmacology 128:191–197

Protais P, Chagraoui A, Arbaoui J (1994): Dopamine receptor antagonist properties of S 14506, 8-OH-DPAT, raclopride and clozapine in rodent. Eur J Pharmacol 271:167–173

Purdon SE, Jones BDW, Stip E, Labelle A, Addington D, Breier A, Tollefson D and the Canadian Collaborative Group for Research on Cognition in Schizophrenia (1998): Olanzapine vs. risperidone and haloperidol in the treatment of cognitive impairment in schizophrenia. Presented at the American College of Neuropsychopharmacology Annual Meeting. Las Croabas, Puerto Rico, December 14–18, 1998

Rasmussen K, Aghajanian GK (1988): Potency of antipsychotics in reversing the effects of a hallucinogenic drug on locus coeruleus neurons correlates with 5-HT_2 binding affinity. Neuropsychopharmacology 1:101–107

Rinaldi-Carmona M, Congy C, Santucci V, Simiand J, Gautret B, Neliat G, Labeeuw B, Le Fur G, Soubrié PG, Breliere JC (1992): Biochemical and pharmacological properties of SR 46349B, a new potent and selective 5-hydroxytryptamine$_2$ receptor antagonists. J Pharmacol Exp Ther 262:759–768

Robertson GS, Matsumura H, Fibiger HC (1994): Induction patterns of fos-like immunoreactivity in the forebrain as predictors of atypical antipsychotic activity. J Pharmacol Exp Ther 271:1058–1066

Rollema H, Lu Y, Schmidt AW (1997): Clozapine increases dopamine release in prefrontal cortex by 5-HT_{1a} receptor activation. Eur J Pharmacol 338:R3–R5

Roth BL, Ciaranello RD, Meltzer HY (1992): Binding of typical and atypical antipsychotic agents with transiently expressed 5-HT_{1c} receptors. J Pharmacol Exp Ther 260:1361–1365

Roth B, Craigo SC, Choudhary MS, Ulue A, Monsma FJ, Shen Y, Meltzer HY, Sibley DR (1994): Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. J Pharmacol Exp Ther 268:1401–1410

Ruotsalainen S, Sirvio J, Jäkälä P, Puumala T, MacDonald E, Riekkin P Sr (1997): Differential effect of three 5-HT receptor antagonists on the performance of rats in attentional and working memory tasks. Eur J Neuropsychopharmacol 7:99–108

Sapolsky RM (1994): The physiological relevance of glucocorticoid endangerment of the hippocampus. Ann NY Acad Sci USA 746:294–304

Schmidt CJ, Sorensen SM, Kehne JH, Carr AA, Palfreyman MG (1995): The role of 5-HT_{2a} receptors in antipsychotic activity. Life Sci 56:2209–2222

Schotte A, Janssen PFM, Gommeren W, Luyten WHML, Van Gompel P, Lesage AS, De Loore K, Leysen JE (1996): Risperidone compared with new and reference antipsychotic drugs: In vitro and in vivo receptor binding. Psychopharmacology 124:57–73

Shipley J (1998): M100907 Phase IIB Trial. Presented at Hoechst Marion Roussel Conference on M100907. West Palm Beach Florida, April 1998

Spampinato U, De Deuwwaerdere P, Caccia S, Lucas G (1998): Opposite role of central 5-HT_{2a} and 5-HT_{2c} receptors subtypes in the control of haloperidol-induced release of dopamine in the rat striatum. Neurosci Abstr 24:48.4

Spampinato U, Stinus L, De Deuwwaerdere P (1997): Effect of dorsal raphe electrical stimulation on stratial and accumbal dopamine release in the rat: Role of 5-$HT_{2a/2c}$ and 5-HT_3 receptors. Neurosci Abstr 23:907.8

Tollefson GD, Sanger TM. (1996): Negative symptoms: A path analytic approach to a double-blind, placebo-and haloperidol-controlled clinical trial with olanzapine. Am J Psychiatry 154:466–474

Truffinet P, Tamminga CA, Fabre LF, Meltzer HY, Riviere ME, Papillon-Downey C (in press): A placebo controlled study of the D_4/5-HT_{2a} antagonist fananserin in the treatment of schizophrenia. Am J Psychiatry

Vaidya VA, Marek GJ, Aghajanian GK, Duman RS (1997): 5-HT_{2a} receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. J Neurosci 17:2785–2795

Varty GB, Higgins GA (1995): Reversal of a dizocilpine-induced disruption of prepulse inhibition of an acoustic startle response by the 5-HT_2 receptor antagonist ketanserin. Eur J Pharmacol 287:201–205

Wadenberg ML (1992): Antagonism by 8-OH-DPAT, but not ritanserin, of catalepsy induced by SCH 23390 in the rat. J Neural Trans 89:49–59

Wadenberg ML, Ahlenius S (1991): Antipsychotic-like profile of combined treatment with raclopride and 8-OH-DPAT in the rat: Enhancement of antipsychotic-like effects without catalepsy. J Neural Trans [Gen Sect] 83:43–53

Wadenberg ML, Hillegaart V (1995): Stimulation of median, but nor dorsal, raphe 5-HT_{1a} autoreceptors by the local application of 8-OH-DPAT reverses raclopride-induced catalepsy in the rat. Neuropharmacology 34:495–499

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REVIEW

Pharmacological and molecular targets in the search for novel antipsychotics

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The recent enthusiasm among clinicians for the so-called 'atypical antipsychotics' has both improved treatment for schizophrenic patients and provided a welcome stimulus for basic research on antipsychotic mechanisms. Even the newer drugs have shortcomings, and research is underway aimed at identifying novel agents with greater efficacy and safety. Much of this effort is directed towards compounds which, in addition to blocking dopamine receptors, also act on other neurotransmitter receptors such as 5-HT_2, 5-HT_{1A} and α_2-adrenergic receptors. However, there is also a large amount of scientific activity seeking to discover and develop selective dopamine receptor subtype antagonists (including compounds which specifically block D_3 or D_4 receptors) or drugs that specifically target the dopamine autoreceptor. Finally, a number of drug development programmes are searching for non-dopaminergic antipsychotics. Drugs that do not have affinity for dopamine receptors but act through neurotensin, sigma or cannabinoid CB_1 receptors or glutamatergic mechanisms are currently being evaluated. If any of these agents prove to have clinical efficacy this may lead to a third generation of antipsychotics. It is likely, however, that the mechanisms of action of such drugs will nevertheless imply the intimate involvement of dopaminergic pathways. © 2000 Lippincott Williams & Wilkins.

Keywords: antipsychotics, dopamine, serotonin, cannabinoid, neurotensin, sigma receptors, glutamate

INTRODUCTION

Since the discovery of the antipsychotic properties of chlorpromazine by Delay and Deniker in 1952, schizophrenia has been treated with a range of classical neuroleptics belonging to diverse chemical classes. While these drugs are undoubtedly effective against the core symptoms of schizophrenia, they also produce a number of severe and unpleasant side effects, including extrapyramidal symptoms (Parkinson-like syndrome, dystonia, akathisia, tardive dyskinesia), sexual dysfunction, orthostatic hypotension, neuroendocrine disturbances, excessive sedation and weight gain, that limit their therapeutic usefulness and reduce the patient's quality of life. Moreover, while most neuroleptics improve the positive symptoms of schizophrenia (such as delusions and hallucinations), the negative symptoms (such as blunted affect and withdrawal) and cognitive deficits often show little improvement. Furthermore, a number of schizophrenic patients are resistant to treatment. These drawbacks of conventional neuroleptics have led to a considerable research effort to discover and develop novel agents that safely treat both the positive and the negative symptoms of schizophrenia while producing few extrapyramidal side effects; these drugs are the so-called 'atypical antipsychotics' (Gerlach and Peacock, 1995; Kinon and Lieberman, 1996; Remington and Kapur, 2000).

In the late 1960s and early 1970s, sulpiride and clozapine provided the first evidence that some antipsychotic drugs could elicit a therapeutic effect in schizophrenic patients with few or no extrapyramidal side effects. These drugs gave impetus to the search for second generation antipsychotics, and these efforts have had some success. In this quest, different, though not mutually exclusive, conceptual paths have been followed. Most of the successful approaches are based on the original dopamine hypothesis of schizophrenia, which proposes that excess dopamine in the brain underlies the positive symptoms of schizophrenia and that blockade of dopamine receptors of the D_2 family is a requisite property for

antipsychotic activity (Carlsson, 1978; Willner, 1997). Major improvements in both antipsychotic and side effect profiles can be obtained through the development of D_2 receptor antagonists, which, in addition, interact with other neurotransmitter systems, in particular, it has been proposed, the serotonergic system (Meltzer, 1996, 1999).

Among compounds that, in addition to having the property of blocking dopamine D_2 receptors, also show affinity for serotonin and other neurotransmitter receptors, risperidone, sertindole, olanzapine, quetiapine and zotepine have recently entered clinical practice, and other compounds, including ziprasidone and iloperidone, are in clinical development (Blin, 1999; Richelson, 1999). However, the apparently opposite approach of developing agents with selectivity for dopamine D_2 receptors as great as or greater than that shown by sulpiride has led to the production of other substituted benzamides, including amisulpride and remoxipride (which was withdrawn because of side effects). There is also considerable interest in the possibility that drugs selective for the D_3 or D_4 dopamine receptor subtypes might have antipsychotic potential (Hadley, 1996; Levant, 1997; Reynolds, 1996).

While the positive symptoms of schizophrenia may result from dopamine neuron hyperactivity (Laruelle *et al.*, 1999), negative symptoms seem to be caused by structural brain damage, evidenced by enlargement of the cerebral ventricles and decreased frontal cortex blood flow (Andreasen *et al.*, 1996). The hypothesis has been put forward that negative symptomatology might involve hypoactivity in cortical dopamine projections (Berman and Weinberger, 1990) and could therefore be treated by increasing dopamine activity (Willner, 1997). This concept has led to the development of preferential dopamine autoreceptor antagonists such as amisulpride (Coukell *et al.*, 1996; Scatton *et al.*, 1997), which differ from the majority of neuroleptics in being clinically effective against both the positive and the primary negative symptoms of schizophrenia (Freeman, 1997; Rein and Turjanski, 1997).

This paper provides an overview of some of the different pharmacological and molecular approaches that have been envisaged so far for the development of novel neuroleptics and that are based on the dopamine hypothesis of schizophrenia. In addition, we discuss some prospective non-dopaminergic targets that are presently being actively pursued.

MULTI-RECEPTOR AGENTS

Serotonin receptors

While a *sine qua non* for antipsychotic action has so far been the blockade of D_2-like receptors, it has been claimed that blockade of additional neurotransmitter receptors, in particular the 5-hydroxytryptamine$_2$ (5-HT_2) subtype of serotonin receptors, may contribute to a low incidence of extrapyramidal side effects and may also play a part in antipsychotic efficacy (particularly with regard to negative symptoms). This line of reasoning was based on the rich and complex pharmacological profile of clozapine, which has affinity for a wide range of neurotransmitter receptors, including dopamine D_1, D_2 and D_4, α_1- and α_2-adrenergic, H_1-histaminergic, muscarinic and several serotonin receptor subtypes (Leysen *et al.*, 1993; Brunello *et al.*, 1995; Ashby and Wang, 1996). It was argued, quite logically, that, as clozapine is an effective antipsychotic with fewer extrapyramidal and other serious side effects (with the exception of agranulocytosis) than traditional neuroleptics, and may in addition be efficacious in patients resistant to other drugs (Kane *et al.*, 1988), this profile is unlikely to result from pharmacological activities, such as D_2 receptor blockade, shared with traditional drugs. Further emphasis has been placed on such arguments by more recent findings showing that, in schizophrenic patients, the proportion of striatal D_2 receptors occupied by therapeutic doses of clozapine (average values in the range 40–60%) seems to be considerably lower than the levels of occupation shown by haloperidol and other classical drugs (> 70%) (Farde *et al.*, 1994; Kapur *et al.*, 1999). It has been claimed, however, that this difference may be an artefact and that the 'real' level of D_2 occupation produced by clinically active doses of clozapine may in fact be about 70% (Seeman and Kapur, 1997).

The blockade of 5-HT_2 receptors (probably the 5-HT_{2A} subtype; Meltzer, 1999) by new generation antipsychotic drugs may be of particular significance. Meltzer and colleagues (1989) proposed that atypical clinical profiles (i.e. antipsychotic activity without extrapyramidal side effects) could be related to the ratio of affinities for 5-HT_{2A} and D_2 receptors. This hypothesis was reinforced by the finding that several drugs other than clozapine, particularly risperidone (Grant and Fitton, 1994), olanzapine (Fulton and Goa, 1997) and sertindole (Sanchez *et al.*, 1991), that have good antipsychotic efficacy and few side effects also have high affinities for 5-HT_{2A} receptors relative to D_2 receptors. It is clear, however, that activity at 5-HT_{2A} receptors is not essential for antipsychotic efficacy as a number of clinically active drugs do not share this property. Therefore, much emphasis was placed on the possibility that blockade of 5-HT_{2A} receptors might prevent D_2 receptor block-

ade in the nigrostriatal system from giving rise to motor side effects (Kapur, 1996). However, as pointed out by Kapur and Remington (1996), it is not necessary to propose such a hypothesis to explain the effects of clozapine, as the lack of extrapyramidal side effects with this drug can be explained more parsimoniously as due simply to the relatively low D_2 receptor occupancy in the striatum. It is therefore necessary to propose another hypothesis, namely that activity at 5-HT_{2A} receptors potentiates antipsychotic effects produced by D_2 receptor occupation. There is indeed some experimental evidence consistent with this idea (Andersson *et al.*, 1995).

However, although clinical studies indicate that antipsychotic doses of quetiapine, like clozapine, are associated with relatively low levels of dopamine receptor occupancy (Küfferle *et al.*, 1997), this is apparently not the case with risperidone and olanzapine (Kapur *et al.*, 1999). Thus, although 5-HT_{2A}-mediated neurotransmission may play a role in mediating the clinical profiles of all these drugs, it seems unlikely that they all act through identical mechanisms. Consistent with this idea is the fact that different drugs also show a variety of differences in their pharmacological characteristics (Arnt and Skarsfeldt, 1998).

It has also been proposed that, in addition to potentiation of the effects produced by D_2 receptor antagonism, blockade of 5-HT_{2A} receptors might itself give rise to antipsychotic effects (Schmidt *et al.*, 1995). Ritanserin, which has antagonist activity at both 5-HT_{2A} and 5-HT_{2C} receptors, has been evaluated in several small-scale clinical trials in schizophrenic patients. In one open study, ritanserin was reported to show antipsychotic efficacy (Wiesel *et al.*, 1994), whereas another trial found antidepressive but not antipsychotic effects (Strauss and Klieser, 1991). When ritanserin was added to neuroleptic treatment in a placebo-controlled, double-blind trial involving 33 schizophrenic patients with predominantly negative symptoms, there was a significant reduction of the symptoms in this class (Duinkerke *et al.*, 1993). Such observations have led to the development and clinical testing of a number of compounds with high affinity and selectivity for 5-HT_{2A} sites (in comparison to D_2 receptors), including MDL 100,907 (Sorensen *et al.*, 1993; Kehne *et al.*, 1996), fananserin (which has additional affinity for dopamine D_4 receptors; Doble *et al.*, 1992) and SR 46349 (Rinaldi-Carmona *et al.*, 1992). Controlled clinical trials with fananserin and MDL 100,907 did not demonstrate clear antipsychotic efficacy, and it remains unclear whether pure 5-HT_{2A} antagonists will find a place in the treatment of schizophrenia (Truffinet *et al.*, 1999). Further clinical research will also be necessary to establish whether 5-HT_{2A} antagonism may be particularly useful in treating negative symptoms.

Although the focus of research on the role of serotonergic mechanisms in antipsychotic drug effects has been on 5-HT_{2A} receptors, activity at 5-HT_{1A}, 5-HT_6 and 5-HT_7 receptors may also be of some significance (Roth *et al.*, 1994; Meltzer, 1999). While the evidence that blocking 5-HT_{2A} receptors can prevent or reduce the motor effects of D_2 blockade is relatively weak (Kapur and Remington, 1996), there are experimental data, both from clinical and animal studies, suggesting that stimulation of 5-HT_{1A} receptors does produce such an effect. Thus, several studies have shown that compounds with 5-HT_{1A} agonist or partial agonist effects, such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and buspirone, can inhibit catalepsy produced by neuroleptics in rats and can enhance other behavioural or biochemical effects of neuroleptics in models believed to reflect antipsychotic activity (Wadenberg and Ahlenius, 1991; Wadenberg, 1996; Prinssen *et al.*, 1996; Ichikawa and Meltzer, 1999). Such results suggest that drugs with combined D_2 antagonist and 5-HT_{1A} agonist properties might show particularly interesting clinical antipsychotic properties. It is worth noting that ziprasidone shows a high affinity for 5-HT_{1A} and D_2 receptors (as well as for 5-HT_{2A}, 5-HT_{2C} and 5-HT_{1D} sites) and has 5-HT_{1A} agonist effects *in vitro* and *in vivo* (Seeger *et al.*, 1995; Sprouse *et al.*, 1999). Ziprasidone has been reported to have good antipsychotic activity against both the positive and the negative symptoms of schizophrenia with few extrapyramidal side effects (Daniel *et al.*, 1999).

Drugs which stimulate 5-HT_{1A} receptors are usually associated with anxiolytic and antidepressant effects rather than antipsychotic action (Barrett and Gleeson, 1991; Thiebot and Martin, 1991). It is therefore particularly intriguing to note that ziprasidone was reported to alleviate depressive symptoms when tested in schizophrenic patients (Daniel *et al.*, 1999). If the clinical profile of ziprasidone is confirmed in further trials and, eventually, in routine clinical use, this might indicate that combined D_2 and 5-HT_{1A} activity could provide particularly good antipsychotic efficacy, although the significance of affinity of ziprasidone for other serotonin receptor subtypes cannot be ruled out entirely. Several other agents with combined D_2 antagonist and 5-HT_{1A} agonist activities have been described, and it will be extremely interesting to observe the antipsychotic

actions of these compounds if they come into clinical use (Rigton *et al.*, 1996; Millan *et al.*, 1998).

Cholinergic mechanisms

While attention has been sharply focused in recent years on potential roles for serotonin receptors in mediating the pharmacological effects of clozapine and other new generation antipsychotics, there is additional evidence that other neurotransmitter receptors for which clozapine has appreciable affinity may also be of importance. Standard clinical practice for many years has involved the treatment of the extrapyramidal side effects of neuroleptics with drugs having antimuscarinic activity. It has also been known for some time that both clozapine and thioridazine have affinity for muscarinic receptors, which might account for the good neurological tolerance of these drugs (Snyder *et al.*, 1974; Josselyn *et al.*, 1997). However, the observation that clozapine increases salivation, whereas salivation is inhibited by anticholinergic drugs, has seemed inconsistent with this idea (but see also Rabinowitz *et al.*, 1996). A possible explanation for this apparent anomaly was provided by the report that clozapine can exert agonist or antagonist effects at different muscarinic receptor subtypes (Zorn *et al.*, 1994). Olanzapine also has significant affinity for muscarinic receptors *in vitro* and *in vivo* (Bymaster *et al.*, 1996). It is also notable, however, that the high affinities of clozapine and olanzapine for muscarinic receptors observed *in vitro* do not always seem to predict high affinities or potencies in *ex vivo* or *in vivo* studies (Schotte *et al.*, 1996; Zhang and Bymaster, 1999).

α-Adrenoceptors

Many antipsychotic drugs, both classical and new generation agents, have appreciable affinities for α-adrenergic receptor sites (Leysen *et al.*, 1993). Traditionally, activity at α_1-adrenoceptors has been thought to be associated with the undesirable actions of antipsychotics such as sedation and cardiovascular side effects (e.g. orthostatic hypotension). It has also been proposed, however, that α_1-adrenoceptor antagonist activity may be of some importance in the mechanism of action of clozapine and, potentially, some other newer drugs. Prinssen and colleagues (1994a, 1994b, 1996) came to this conclusion on the basis of their results showing interactions between α_1-adrenergic agonists and antagonists, and haloperidol and clozapine, in the paw test and a conditioned avoidance procedure, two methods believed to predict clinical antipsychotic effects. Other studies have reported antipsychotic-like behavioural effects of α_1-adrenoceptor antagonists. For example, prazosin has been reported to block amphetamine-induced hyperactivity in rodents (Arnt, 1995; Darracq *et al.*, 1998).

In addition, Nutt *et al.* (1997) suggested that the affinity of clozapine for α_2-adrenoceptors (approximately the same as the drug's affinity for D_2 sites) may be of importance, noting that α_2-adrenoceptor mechanisms in the frontal cortex may be of particular significance in mediating cognitive processes. It has also been reported that the α_2-adrenoceptor antagonist idazoxan can potentiate the antipsychotic effect of fluphenazine (Litman *et al.*, 1993). Several recent experimental studies also produced results consistent with the view that α_2-adrenoceptor antagonism produced by clozapine may be responsible either for the lack of extrapyramidal side effects or for the good antipsychotic efficacy of this drug. For example, clozapine can actually inhibit the catalepsy produced by the classical neuroleptic loxapine, an effect which may be related to α_2-adrenoceptor blockade. However, McAllister and Rey (1999) have recently presented results suggesting that this effect is due to the anticholinergic properties of clozapine. Nevertheless, certain behavioural effects of haloperidol and chlorpromazine can be potentiated by idazoxan, and it has also been reported that combined administration of clozapine and clonidine gives rise to a haloperidol-like behavioural profile in an electrical self-stimulation procedure (Hertel *et al.*, 1999; Montgomery *et al.*, 1999). Raclopride-induced dopamine release in the frontal cortex is also potentiated by idazoxan (Hertel *et al.*, 1999). These results, although limited, suggest that combined blockade of D_2 and α_2 receptors may produce particularly effective and well-tolerated antipsychotics.

COMPOUNDS ACTING SELECTIVELY AT DOPAMINE RECEPTORS

Selective dopamine receptor subtype antagonists

Five dopamine receptor subtypes have so far been identified. They fall into two classes: the D_1/D_5 family and the D_2-like family (which includes D_2, D_3 and D_4 subtypes). All five receptor subtypes have been envisaged as possible targets for atypical antipsychotic drugs.

Josselyn, Miller and Beninger (1997) proposed that clozapine is an especially effective antipsychotic because it acts directly at D_1 receptors, and that D_1 receptor blockade may be the final common pathway for the actions of all antipsychotic drugs. This hypothesis suggests that selective D_1 antagonists would be of particular interest clinically. The search for selective D_1 receptor antagonists led to the discov-

ery of SCH-39166, a compound that in preclinical studies showed efficacy in models predictive of antipsychotic activity (inhibition of conditioned avoidance responses in monkeys and of apomorphine-induced climbing behaviour in the mouse), at doses largely dissociated from those that induced catalepsy and that did not elevate prolactin levels (Chipkin *et al.*, 1988). Clinical trials with SCH-39166, however, failed to demonstrate efficacy on the positive or negative symptoms of schizophrenia (Karlsson *et al.*, 1995), and the clinical antipsychotic effect of a second D_1 antagonist, NNC 01-0686, was also not very convincing (Karle *et al.*, 1995). Taken together, these results do not encourage the view that further development of D_1 antagonists would lead to better antipsychotic agents.

Recent positron emission tomography (PET) studies have revealed a reduction in the density of D_1 receptors in the prefrontal cortex in schizophrenia that was related to the severity of the negative symptoms and cognitive deficits (Okubo *et al.*, 1997). This suggests that further reduction of an already endogenously reduced D_1 function via D_1 receptor blockade would not be expected to ameliorate, but rather to worsen, the negative symptoms of schizophrenia. Whether partial or full agonists at D_1 receptors would be useful for treating the negative or cognitive symptoms of schizophrenia is worth exploring.

Among the D_2-like receptors, the D_4 and D_3 receptors have attracted most attention because of their exclusive localization in areas of the brain that may be relevant to schizophrenia (the limbic and cortical regions). The discoveries that clozapine is particularly potent at blocking D_4 receptors, and that a wide range of neuroleptics have a high affinity for the D_4 receptor, which is negatively correlated to their ability to induce catalepsy, have reinforced interest in the development of pure D_4 receptor antagonists (Reynolds, 1996; Seeman *et al.*, 1997).

This approach is actively being pursued by a number of pharmaceutical companies, which have identified very potent (nanomolar range) and selective (selectivity ratio of D_4 to D_2 ranging from 200- to 2000-fold) D_4 receptor antagonists such as L-745,870, NGD 94-1, U-101387, YM-50001, PD17185 and CP293019. These compounds are generally inactive in traditional behavioural models predictive of antipsychotic activity or side effects (Hadley, 1996; Bristow *et al.*, 1997a,b). However, some D_4 antagonists have been shown to reverse apomorphine- or amphetamine-induced hyperactivity at relatively high doses (Hadley, 1996) though this might be a D_2 receptor mediated effect. Interestingly, NGD 94-1 and PD17185 also reverse apomorphine-induced disruption of prepulse inhibition of the acoustic startle response in rats, a test which mirrors impairment of attentional function and information processing in schizophrenic patients, and NGD 94-1 antagonized cognitive deficits in monkeys produced by phencyclidine (Tallman, 1998; Jentsch *et al.*, 1999).

Unfortunately, recent double-blind clinical trials performed with L-745,870 and with the mixed D_4/5-HT_2 antagonist fananserin in schizophrenic patients have been disappointing, since they failed to demonstrate any improvement in positive or negative symptoms compared with placebo. With L-745,870 the dose was chosen as that which, on the basis of indirect estimates, was expected to occupy 90% of cerebral D_4 receptors, which excludes the possibility that the drug dosage was insufficient (Kramer *et al.*, 1997; Truffinet *et al.*, 1999).

Following the proposal that D_3 receptors may be of particular significance for schizophrenia and antipsychotic drug actions (Sokoloff *et al.*, 1990), several selective D_3 antagonists and various compounds with high potency (nanomolar range) for D_3 receptors and moderate or high levels of selectivity for D_3 compared with D_2 receptors have been described, including nafadotride, PNU 99194A, S-14287 and GR-10369 (Sautel *et al.*, 1995; Millan *et al.*, 1995; Murray *et al.*, 1995; Haadsma-Svensson and Svensson, 1998). Nafadotride antagonizes apomorphine-induced climbing behaviour and hypothermia (tests predictive of antipsychotic activity), but catalepsy was induced at similar doses (Sautel *et al.*, 1995; Audinot *et al.*, 1998). This suggests that the selectivity of nafadotride for D_3 compared with D_2 receptors is not sufficient to allow separation between the antipsychotic and extrapyramidal effects. An interesting feature of this compound, however, is its ability at low doses to facilitate place conditioning induced by food in rats (a prohedonic-like effect) (Chaperon and Thiebot, 1996), which suggests potential in the treatment of the negative symptoms of schizophrenia. (+)S-14297 was reported to antagonize hypothermia induced by the D_3-preferring agonist 7-0H-DPAT in rats at relatively low doses, but did not induce catalepsy or elevate plasma prolactin levels (Millan *et al.*, 1995; Audinot *et al.*, 1998). This compound has also been shown to block haloperidol-induced catalepsy without affecting the disruption of conditioned avoidance responding produced by the neuroleptic (Millan *et al.*, 1997). This favourable pharmacological profile suggests that (+)S-14297 could possess antipsychotic activity without inducing extrapyramidal or

neuroendocrine side effects in schizophrenic patients. However, as research progresses with the few selective D_3 antagonists so far available, it is becoming clear that no prototypical D_3 antagonist pharmacological profile is emerging (Audinot *et al.*, 1998; Clifford and Waddington, 1998). Furthermore, as shown by experiments with D_3 receptor knock-out mice, certain behavioural effects of at least some of these compounds seem not to be mediated through dopamine D_3 receptors (Boulay *et al.*, 1999).

It appears that, among the different dopamine receptor subtypes possibly implicated in antipsychotic drug action, the D_2 and D_3 receptors are currently the leading candidates. Nevertheless, the relative role of these two receptors remains to be established by clinical studies with new selective D_2 and D_3 antagonists. It is not yet clear whether blockade of D_3 receptors alone will be sufficient to achieve antipsychotic effects, and more effective antipsychotic medication may require action at both subtypes of D_2-like receptors.

Dopamine autoreceptors

Dopamine receptors are located not only on dopaminoceptive postsynaptic elements, but also presynaptically on dopaminergic neurons. It is now well established that presynaptic D_2 and D_3 receptors are involved in the local regulation of the release of dopamine from nerve terminals through a negative feedback mechanism, and somatodendritic D_2/D_3 autoreceptors are involved in the control of the firing of dopaminergic neurons (Langer and Lehmann, 1988). Therefore, another approach to developing atypical neuroleptics involves targeting presynaptic D_2/D_3 receptors. Extensive clinical trials with the benzamide derivative amisulpride, a blocker of D_2/D_3 autoreceptors, have revealed a clear-cut efficacy of this drug at low doses on primary negative symptoms and at higher doses on both the positive and negative symptoms of schizophrenia, with a low propensity to induce neurological side effects and a very good general tolerance (Coukell *et al.*, 1996; Freeman, 1997; Rein and Turjanski, 1997). This remarkable antipsychotic action of amisulpride appears to result from its pharmacological profile. Binding studies have shown that amisulpride is a specific D_2 and D_3 receptor antagonist, with a seven-fold selectivity for the native D_3 compared with the D_2 receptor *in vitro* (Schoemaker *et al.*, 1997a). A higher D_3 versus D_2 selectivity ratio was found in *in vivo* and *ex vivo* binding studies in mice and rats (Scatton *et al.*, 1997; Schoemaker *et al.*, 1997b). An important feature of amisulpride is its ability to block presynaptic D_2/D_3 autoreceptors *in vitro* at nanomolar concentrations (antagonism of 7-OH-DPAT induced decrease in electrically evoked release of ^{3}H-dopamine from rat nucleus accumbens slices) and *in vivo* at relatively low doses. Amisulpride is 17 times more selective *in vivo* for presynaptic versus postsynaptic D_2/D_3 receptors. This property of amisulpride allows it to enhance dopaminergic transmission in certain brain areas at low doses, and may account for its efficacy on the negative symptoms of schizophrenia, which have been suggested to reflect a state of decreased cortical dopaminergic function (Berman and Weinberger, 1990). Behavioural data in rats demonstrating prohedonic-like effects of amisulpride at low doses in a food-induced place conditioning procedure, and anti-anhedonic-like effects in a chronic mild stress model, are in line with this hypothesis (Guyon *et al.*, 1993; Papp and Wieronska, 2000). Moreover, low dopamine-autoreceptor-selective doses of amisulpride markedly increase glucose use (an index of integrated functional activity) in several cortical areas of the rat, including the prefrontal, cingulate, temporal and orbital cortices (Cudennec *et al.*, 1997), suggesting that amisulpride, by enhancing dopaminergic transmission in cortical areas, may be able to correct the hypofrontality seen in schizophrenic patients. The question of how amisulpride, or other agents, can show apparent selectivity for presynaptic dopamine receptors, since the nature of the presynaptic and postsynaptic receptors has not been shown to be fundamentally different, has yet to be clearly answered. One might speculate, however, that this property relates to a particularly important presynaptic location of D_3 receptors (for example see Kreiss *et al.*, 1995).

At higher doses, amisulpride also blocks postsynaptic D_2/D_3 receptors, with a preferential effect in the limbic system (Schoemaker *et al.*, 1997a), and antagonizes dopamine-dependent behaviours linked to excess dopamine transmission in the limbic system (apomorphine-induced climbing and amphetamine-induced hyperlocomotion) without inducing catalepsy in the rodent (Perrault *et al.*, 1997). After repeated treatment, amisulpride also reduces the number of spontaneously active dopamine neurons in the ventral tegmental area, but not in the substantia nigra (Di Giovanni *et al.*, 1998). The preferential blockade of dopaminergic transmission by amisulpride in the limbic system may account for its ability to prevent the florid symptoms of schizophrenia with minimal extrapyramidal side effects.

Thus, the dual efficacy of amisulpride against the primary negative and positive schizophrenic symp-

toms may be attributable to the relative predominance of presynaptic or postsynaptic actions, resulting in a net increase in dopaminergic function at low doses and in a net decrease in dopamine transmission at higher doses. This emphasizes the importance of the dopamine autoreceptor antagonist approach for the treatment of both forms of schizophrenia. It should also be pointed out, however, that like most antipsychotics (but not clozapine and olanzapine, for reasons which are not yet clear), amisulpride does give rise to increases in plasma prolactin levels (Dickson and Glazer, 1999; Grunder *et al.*, 1999b)

Despite the evident success of amisulpride, it is not clear whether other agents with identical mechanisms of action are currently being developed. However, at least one drug, aripiprazole, that may act through presynaptic and postsynaptic dopamine receptors is being tested clinically. Like amisulpride, aripiprazole blocks the effects of apomorphine but does not produce catalepsy except at much higher doses (Semba *et al.*, 1995). It shows a high affinity for dopamine D_2 receptors, with a much lower affinity for serotonin receptors (Lawler *et al.*, 1999). However, electrophysiological, biochemical and behavioural experiments indicate that aripiprazole, while blocking postsynaptic D_2 receptors, may actually have agonist activity at presynaptic sites (Kikuchi *et al.*, 1995; Semba *et al.*, 1995; Fujikawa *et al.*, 1996). Little information is available on the clinical profile of aripiprazole, but an early report suggested that the drug may be active against both the positive and the negative symptoms of schizophrenia (Toru *et al.*, 1994).

BEYOND THE DOPAMINE HYPOTHESIS OF SCHIZOPHRENIA

As stressed in the Introduction, most of the current approaches for the development of antipsychotic agents are derived from the dopamine hypothesis of schizophrenia. However, it is possible that the abnormalities of dopamine transmission in schizophrenia may be secondary to alterations in other neuronal systems that have intimate interactions with the dopamine systems.

Neurotensin receptor agonists and antagonists

Neurotensin is a tridecapeptide which is colocalized with dopamine in dopaminergic neurons that project specifically to the prefrontal cortex and nucleus accumbens, and it acts as a modulator of dopaminergic transmission in various brain areas (Kasckow and Nemeroff, 1991; Rostene *et al.*, 1997). It is also of interest here to note that other researchers have proposed that the effects of currently used antipsychotic drugs may be mediated through neurotensin-containing neurons, and that neurotensin agonists might show antipsychotic activity (Liegeois *et al.*, 1995; Kinkead *et al.*, 1999). In line with this hypothesis, a recent study reported that the neurotensin agonist PD149163 produced antipsychotic-like effects (i.e. antagonized the effect of amphetamine) on the prepulse inhibition of the startle response in rats (Feifel *et al.*, 1999), and that blockade of neurotensin transmission with SR 142948 prevents the behavioural effects of haloperidol in the prepulse inhibition of the acoustic startle reflex and latent inhibition procedures (Kinkead *et al.*, 1999).

In apparent contradiction to this approach, there is also evidence to indicate that neurotensin antagonists may have antipsychotic potential. Neurotensin has been shown to increase the firing of dopaminergic neurons and the release of dopamine in the nucleus accumbens and striatum *in vitro* and *in vivo*. Moreover, local injections of neurotensin into the ventral tegmentum increase locomotor activity and have been reported to potentiate the disruption of prepulse inhibition produced by dopamine agonists (Feifel *et al.*, 1997). Very potent and selective non-peptidic neurotensin receptor antagonists such as SR 48692 and SR 142948 have been identified (Gully *et al.*, 1993, 1997). When given chronically, SR 48692 decreases the spontaneous electrical activity of dopaminergic neurons in the ventral tegmentum but not in the substantia nigra, and selectively decreases basal release of dopamine in the nucleus accumbens (Santucci *et al.*, 1997; Azzi *et al.*, 1998). Moreover, SR 48692 antagonizes the behavioural effects of psychostimulants (Betancur *et al.*, 1998). Neurotensin antagonists may thus represent a new type of antipsychotic drug that acts by preventing excessive dopaminergic neuron activity via a blockade of a pathologically enhanced neurotensin tone on these neurons. Phase II clinical trials with SR 48692 are currently underway in schizophrenia.

Glutamate receptor agonists

Glutamate is the major excitatory neurotransmitter in the central nervous system. Several lines of evidence have suggested that a dysregulation of glutamatergic efferent projections may be involved in the pathophysiology of schizophrenia (Carlsson and Carlsson, 1990). Phencyclidine, an antagonist of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor, is known to induce a range of behavioural effects very similar to both positive and negative schizophrenic symptoms. These include thought dis-

orders, delusions, affective blunting and cognitive dysfunction. Phencyclidine has also been reported to elicit a recrudescence of psychosis in stabilized schizophrenic patients (Allen and Young, 1978). Moreover, long-term administration of phencyclidine in monkeys causes a reduction in dopamine utilization in the dorsolateral prefrontal cortex (reminiscent of the hypofunction of cortical dopamine neurons suspected to occur in negative schizophrenia) and enduring cognitive deficits that were improved by clozapine treatment (Jentsch *et al.*, 1997). Post mortem studies have reported abnormalities in glutamatergic markers in the frontal lobe of schizophrenic individuals, which may underlie NMDA receptor hypofunction (Nishikawa *et al.*, 1994). These findings have contributed to the development of a hypoglutamatergic hypothesis of schizophrenia and suggest that NMDA receptor agonists may possess antipsychotic potential. Whether compounds that enhance glutamatergic transmission produce antipsychotic effects is beginning to be investigated clinically. Glycine or D-serine, by stimulating the glycine modulatory site present on the NMDA receptor, would be expected to enhance glutamatergic transmission and to improve schizophrenic symptoms. Administration of high doses of glycine and D-cycloserine (a glycine site partial agonist), alone or in combination with neuroleptics, has been claimed to ameliorate negative schizophrenic symptoms (Javitt *et al.*, 1994; Goff *et al.*, 1995), but results with the glycine prodrug milacemide have been inconsistent (Rosse *et al.*, 1990).

It has also been proposed that compounds that enhance glutamatergic neurotransmission through allosteric modulation of the AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxatepropionic acid) subtype of glutamate receptors (called ampakines) may have utility in treating schizophrenia (Johnson *et al.*, 1999). One such compound has been found to inhibit amphetamine-induced hyperactivity in rats (Larson *et al.*, 1996). Similarly, there is evidence that metabotropic glutamate receptors may be implicated in schizophrenia and antipsychotic drug action (Grauer and Marquis, 1999; Richardson-Burns *et al.*, 2000).

Sigma receptors

Sigma receptors were first proposed by Martin and colleagues (1976) as a subtype of opiate receptors that mediate the behavioural effects of SKF 10,047 (*N*-allylnormetazocine). These effects include stimulant actions in experimental animals and symptoms reminiscent of schizophrenia, such as depersonalization, in human subjects. However, there was initially some confusion between sigma receptors and the phencyclidine-binding site associated with the NMDA subtype of glutamate receptors, for which SKF 10,047 has affinity. Nevertheless, there has been a considerable research effort aimed at investigating the functional significance of sigma receptors (Walker *et al.*, 1990). The idea that these receptors provide an appropriate target for novel antipsychotic drug development remains strong, particularly as several classical antipsychotics, including haloperidol, have particularly high affinities for these receptors (Tam and Cook, 1984; Deutsch *et al.*, 1988; Snyder and Largent, 1989). Moreover, the recent demonstration of an association between polymorphisms in the $sigma_1$ receptor gene and schizophrenia suggests that this receptor may play a role in the pathogenesis of schizophrenia (Ishiguro *et al.*, 1998).

In recent years a number of compounds with affinity for sigma receptors but not for dopamine D_2 receptors have been described. Some of these agents, including NE-100, MS-377 and SR 31742A, are reported to block certain behavioural actions of phencyclidine or dopaminomimetics, which might indicate antipsychotic effects (Okuyama *et al.*, 1994; Takahashi *et al.*, 1999; Poncelet *et al.*, 1993). Moreover, SR 31742A reduces conditioned avoidance responding in the rat. Interestingly these compounds do not produce catalepsy. There is also evidence that sigma ligands, including SA 4503, E-5842 and SR 31742A when given acutely, preferentially increase the firing of dopaminergic neurons in the limbic system (Poncelet *et al.*, 1993; Minabe *et al.*, 1999; Sanchez-Arroyas and Guitart, 1999). Moreover, when given subacutely, SR 31742A, like atypical neuroleptics, preferentially decreases the number of spontaneously active dopaminergic cells in the ventral tegmentum compared with the substantia nigra (Poncelet *et al.*, 1993). It is likely, therefore, that these compounds reduce dopaminergic transmission, possibly through potentiation of NMDA receptor-mediated neurotransmission, which might predict clinical antipsychotic effects (Debonnel and de Montigny, 1996; Liang and Wang, 1998). It has also been proposed, however, that certain motor side effects of classical neuroleptics may be mediated through sigma binding sites (Walker *et al.*, 1988; Jeanjean *et al.*, 1997).

At least three compounds with affinity for sigma receptors, BMY 14802, eliprodil and panamesine, have already been tested in schizophrenic patients. In small trials neither BMY 14802 nor eliprodil was found to have efficacy against positive symptoms, but there was some evidence that eliprodil might alleviate negative symptoms (Garreau *et al.*, 1992;

Gerwitz *et al.*, 1994; Modell *et al.*, 1996). Panamesine was found to have some antipsychotic activity but also gave rise to extrapyramidal side effects. It seems likely that these effects were produced by a metabolite that, unlike panamesine itself, acts directly at dopamine D_2 receptors (Frieboes *et al.*, 1997; Grunder *et al.*, 1999a; Huber *et al.*, 1999). Other sigma ligands, including SR 31742A, are currently in clinical trials, and more information about the antipsychotic potential of these agents should soon become available.

Cannabinoid (CB_1) receptor antagonists

Another original approach to the development of antipsychotic drugs involves agents whose actions may be mediated by endogenous cannabinoid mechanisms. It is believed that many of the pharmacological effects of marijuana may be mediated through specific receptors (CB_1 and CB_2), and it has been proposed that dysfunction of the endogenous cannabinoid system may play a role in the production of at least some of the symptoms of schizophrenia (Emrich *et al.*, 1997). The recent finding that levels of the endogenous cannabinoids anandamide and palmitoylethanolamide were increased in the cerebrospinal fluid of schizophrenic patients seems consistent with this hypothesis (Leweke *et al.*, 1999). Furthermore, cannabinoid receptor agonists such as delta9-THC have been found to activate mesolimbic dopaminergic neurons through an action on CB_1 receptors (French, 1997; Gessa *et al.*, 1998). These observations have led to the suggestion that compounds with CB_1 antagonist properties might produce antipsychotic effects (Alonso *et al.*, 1999).

A selective CB_1 antagonist, SR 141716, has been discovered, and its pharmacology analysed in some detail (Rinaldi-Carmona *et al.*, 1995, 1996). This compound antagonizes most of the pharmacological effects of cannabinoid receptor agonists (for example see Costa *et al.*, 1999), and has also been found to inhibit the stimulant action of amphetamine and cocaine in habituated but not in non-habituated gerbils, a profile also shown by clozapine (Poncelet *et al.*, 1999). In addition, SR 141716 increases Fos-like immunoreactivity in limbic and cortical regions of rat brain, an action it shares with a number of the newer antipsychotics (Alonso *et al.*, 1999). These effects suggest that SR 141716 may show clinical antipsychotic activity, and clinical trials are currently underway to test this hypothesis.

CONCLUSIONS

The recent introduction of several so-called atypical or new generation antipsychotic drugs seems to have significantly improved the treatment and quality of life of schizophrenic patients. The interest generated by these drugs has also had the effect of stimulating research, so that a large number of drug discovery and development programmes searching for novel antipsychotics are currently underway. Many of these are based on neurochemical mechanisms modulated by clozapine and other multi-receptor drugs, in the belief that these mechanisms may play important roles in the efficacy and good side effect profiles of these drugs. However, as noted several times in this paper, all the currently used drugs, and many still in research and development, block dopamine receptors. Thus, although it has been necessary to develop more sophisticated versions of the dopamine hypothesis of schizophrenia since it was first proposed over two decades ago (for example see Joyce, 1993; Willner, 1997), evidence still suggests that dopamine receptors play a major role in mediating the effects of antipsychotic drugs.

It is possible to identify three main threads running through antipsychotic drug research at present. The first consists in the search for agents that, in addition to showing antagonistic activity at dopamine D_2 receptors, also act at other neurotransmitter receptors. Following the lead shown by clozapine, risperidone, olanzapine and some other drugs, the emphasis has been on affinity for 5-HT_{2A} receptors. However, it seems likely that more novel compounds targeting other receptors, perhaps 5-HT_{1A} or α_2-adrenergic, might produce drugs with somewhat different profiles, and perhaps advantages in at least some patients.

The second major area of research focuses on drugs acting selectively at dopamine receptors. The pharmacological and clinical profiles of amisulpride have confirmed that dopamine-selective drugs can show good efficacy and safety. With the current enthusiasm for research on dopamine receptor subtypes it is clear that additional, selectively dopaminergic drugs will be tested clinically in the near future. Such studies may help to clarify the exact role of dopamine and different receptor subtypes in both drug action and disease processes.

The third research thread, which is surely the most speculative but also the most innovative, involves the development of antipsychotics without affinity for dopamine receptors. Over many years researchers have hoped that the development of non-dopaminergic antipsychotics would be possible and that such drugs would eliminate the side effects associated with dopamine receptor blockade, such as extrapyramidal symptoms and increases in prolactin

levels. Such drugs have yet to be found active. However, several interesting approaches are currently being pursued, including sigma receptor ligands, neurotensin and CB_1 receptor antagonists, as well as glutamate receptor agonists, and it can be hoped that some will prove to be clinically efficacious. If they are, this might truly represent a new approach to the treatment of schizophrenia. It should be noted, however, that the logic of all these research and development programmes lies in interactions between novel mechanisms and dopaminergic pathways. It is debatable, therefore, whether even these original drug targets will lead to what could be considered truly non-dopaminergic antipsychotic drugs.

REFERENCES

Allen RM, Young SI (1978). Phencyclidine-induced psychosis. *Am J Psychiatry* **135**:1081–1084.

Alonso R, Voutsinos B, Fournier M, Labie C, Steinberg R, Souilhac J, Le Fur G, Soubrie P (1999). Blockade of cannabinoid receptors by SR141716 selectively increases Fos expression in rat mesocorticolimbic areas via reduced dopamine D_2 function. *Neuroscience* **91**:607–620.

Andersson JL, Nomikos GG, Marcus M, Hertel P, Mathe JM, Svensson TH (1995). Ritanserin potentiates the stimulatory effects of raclopride on neuronal activity and dopamine release selectively in the mesolimbic dopaminergic system. *Naunyn-Schmiedeberg's Arch Pharmacol* **352**:374–385.

Andreasen N, O'Leary D, Arndt S, Cizadlo T, Nopoulos P, Flaum M (1996). Neural substrates of frontal function in schizophrenia. In: *Dopamine Disease States*. Beninger RT, Palomo T, Archer T (editors). Madrid: Editorial Cym, pp. 523–536.

Arnt J (1995). Differential effects of classical and newer antipsychotics on the hypermotility induced by two dose levels of D-amphetamine. *Eur J Pharmacol* **283**:55–62.

Arnt J, Skarsfeldt T (1998). Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. *Neuropsychopharmacology* **18**:63–101.

Ashby CR, Wang RY (1996). Pharmacological actions of the atypical antipsychotic drug clozapine: a review. *Synapse* **24**:349–394.

Audinot V, Newman-Tancredi A, Gobert A, Rivet JM, Brocco M, Lejeune F, Gluck L, Deposte I, Bervoets K, Dekeyne A, Millan MJ (1998). A comparative in vitro and in vivo pharmacological characterization of the novel dopamine D_3 receptor antagonist (+)-S14297, nafadotride, GR 103,691 and U 99194. *J Pharmacol Exp Ther* **287**:187–197.

Azzi M, Betancur C, Sillaber I, Spangel P, Rostene W, Berod A (1998). Repeated administration of the neurotensin receptor antagonist SR 48692 differentially regulates mesocortical and mesolimbic dopaminergic systems. *J Neurochem* **71**:1158–1167.

Barrett JE, Gleeson S (1991). Anxiolytic effects of 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines: conflict and drug discrimination studies. In: *5-HT_{1A} Agonists, 5-HT_3 Antagonists and Benzodiazepines: Their Comparative Behavioural Pharmacology*. Rodgers RJ, Cooper SJ (editors). Chichester: Wiley, pp. 59–105.

Berman KF, Weinberger DR (1990). Prefrontal dopamine and defect symptoms in schizophrenia. In: *Negative Schizophrenic Symptoms: Pathophysiology and Clinical Implications*. Greden JF, Tandon R (editors). London: American Psychiatry Press, pp. 81–95.

Betancur C, Cabrera R, De Kloet E, Pelaprat D, Rostène W (1998). Role of endogenous neurotensin in the behavioral and neuroendocrine effects of cocaine. *Neuropsychopharmacology* **19**:322–332.

Blin O (1999). A comparative review of new antipsychotics. *Can J Psychiatry* **44**:235–244.

Boulay D, Depoortere R, Rostene W, Perrault G, Sanger DJ (1999) Dopamine D_3 receptor agonists produce similar decreases in body temperature and locomotor activity in D_3 knock-out and wild-type mice. *Neuropharmacology* **38**:555–565.

Bristow LJ, Collinson N, Cook GP, Curtis N, Freedman SB, Kulagowski JJ, Leeson PD, Patel S, Ragan CI, Ridgill M, Saywell KL, Tricklebank MD (1997a). L-745,870, a subtype selective dopamine D_4 receptor antagonist does not exhibit a neuroleptic-like profile in rodent behavioral tests. *J Pharmacol Exp Ther* **283**:1256–1263.

Bristow LJ, Kramer MS, Kulagowski J, Patel S, Ragon CI, Seabrook GR (1997b). Schizophrenia and L-745,870, a novel dopamine D_4 receptor antagonist. *Trends Pharmacol Sci* **18**:186–188.

Brunello N, Masotto C, Steardo L, Markstein R, Racagni G (1995) New insights into the biology of schizophrenia through the mechanism of action of clozapine. *Neuropharmacology* **13**:177–213.

Bymaster FP, Hemrick-Luecka SK, Perry KW, Fuller RW (1996). Neurochemical evidence for antagonism by olanzapine of dopamine, serotonin, α_1-adrenergic and muscarinic receptors in vivo in rats. *Psychopharmacology* **124**:87–94.

Carlsson A (1978). Antipsychotic drugs, neurotransmitters and schizophrenia. *Am J Psychiatry* **135**:164–173.

Carlsson M, Carlsson A (1990). Interactions between glutamatergic and monoaminergic systems within the basal ganglia: implications for schizophrenia and Parkinson's disease. *Trends Neurosci* **13**:272–276.

Chaperon F, Thiebot MH (1996). Effects of dopaminergic D_3-receptor-preferring ligands on the acquisition of place conditioning in rats. *Behav Pharmacol* **7**:105–109.

Chipkin RE, Iorio LC, Coffin VL, McQuade RD, Berger JG, Barnett A (1988). Pharmacological profile of SCH-39166: a dopamine D_1 selective benzonaphthazepine with potential antipsychotic activity. *J Pharmacol Exp Ther* **247**:1093–1102.

Clifford JJ, Waddington JL (1998). Heterogeneity of behavioural profile between three new putative selective D_3 dopamine antagonists using an ethologically based approach. *Psychopharmacology* **136**:284–290.

Costa B, Vailati S, Colleoni M (1999). SR 141716A, a cannabinoid receptor antagonist, reverses the behavioural effects of anandamide-treated rats. *Behav Pharmacol* **10**:327–331.

Coukell AJ, Spencer CM, Benfield P (1996). Amisulpride. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the management of schizophrenia. *CNS Drugs* **6**:237–256.

Cudennec A, Fage D, Benavides J, Scatton B (1997). Effect of amisulpride, an atypical antipsychotic which blocks preferentially presynaptic dopamine autoreceptors, on integrated functional cerebral activity in the rat. *Brain Res* **768**:257–265.

Daniel DG, Zimbroff DL, Potkin SG, Reeves KR, Harrigan EP, Lakshminarayanan M (1999). Ziprasidone 80 mg/day and 160 mg/day in the acute exacerbation of schizophrenia and schizoaffective disorder: a 6-week placebo controlled trial. *Neuropsychopharmacology* **20**:491–505.

Darracq L, Blanc G, Glowinski J, Tassin JP (1998). Importance of

the noradrenaline-dopamine coupling in the locomotor activating effects of D-amphetamine. *J Neurosci* **18**:2729–2739.

Debonnel G, de Montigny C (1996). Modulation of NMDA and dopaminergic neurotransmissions by sigma ligands: possible implications for the treatment of psychiatric disorders. *Life Sci* **58**:721–734.

Deutsch SI, Weizman A, Goldman ME, Morihisa JM (1988). The sigma receptor: a novel site implicated in psychosis and antipsychotic drug efficacy. *Clin Neuropharmacol* **11**:105–119.

Dickson RA, Glazer WM (1999). Neuroleptic-induced hyperprolactinemia. *Schizophr Res* **35**:S75–S86.

Di Giovanni G, Di Mascio M, Di Matteo V, Esposito E (1998). Effects of acute and repeated administration of amisulpride, a dopamine D_2/D_3 receptor antagonist, on the electrical activity of midbrain dopaminergic neurons. *J Pharmacol Exp Ther* **287**:51–57.

Doble A, Girdlestone D, Piot O, Allam D, Betschart J, Boireau A, Dupuy A, Gueremy C, Menager J, Zundel JL, Blanchard JC (1992). Pharmacological characterization of RP 62203, a novel 5-hydroxytryptamine 5-HT_2 receptor antagonist. *Br J Pharmacol* **105**:27–36.

Duinkerke SJ, Botter PA, Jansen AA, van Dongen PA, van Haaften AJ, Boom AJ, van Laarhoven JH, Busard HL (1993). Ritanserin, a selective 5-$HT_{2/1C}$ antagonist, and negative symptoms in schizophrenia. A placebo-controlled double-blind trial. *Br J Psychiatry* **163**:451–455.

Emrich HM, Leweke FM, Schneider U (1997). Towards a cannabinoid hypothesis of schizophrenia: cognitive impairments due to dysregulation of the endogenous cannabinoid system. *Pharmacol Biochem Behav* **56**:803–807.

Farde L, Nordstrom AL, Nyberg S, Halldin C, Sedvall G (1994). D_1-, D_2-, and $5HT_2$-receptor occupancy in clozapine-treated patients. *J Clin Psychiatry* **55**(suppl B):67–69.

Feifel D, Roza TL, Robeck SL (1997). Pro-dopamine effects of neurotensin on sensorimotor gating deficits. *Peptides* **18**:1457–1460.

Feifel D, Reza TL, Wustrow DJ, Davis MD (1999). Novel antipsychotic-like effects on prepulse inhibition of startle produced by a neurotensin agonist. *J Pharmacol Exp Ther* **288**:710–713.

Freeman HL (1997). Amisulpride compared with standard neuroleptics in acute exacerbations of schizophrenia: three efficacy studies. *Int Clin Psychopharmacol* **12**(suppl 2):S11–S18.

French ED (1997). Delta9-tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opiod receptors. *Neurosci Lett* **226**:159–162.

Frieboes RM, Murck H, Wiedemann K, Holsboer F, Steiger A (1997). Open clinical trial on the sigma ligand panamesine in patients with schizophrenia. *Psychopharmacology* **132**:82–88.

Fujikawa M, Nagashima M, Inoue T, Yamada K, Furukawa T (1996). Partial agonist effects of OPC-14597, a potential antipsychotic agent, on yawning behavior in rats. *Pharmacol Biochem Behav* **53**:903–909.

Fulton B, Goa KL (1997). Olanzapine: a review of its pharmacological properties and therapeutic efficacy in the management of schizophrenia and related psychoses. *Drugs* **53**:281–298.

Garreau M, Giroux C, L'Heritier C, Coupez M, Eich F, Morselli PL (1992). Pilot study on the effect of SL82.0715 in psychotic syndromes. *Clin Neuropharmacol* **15**(suppl 1):699A–700A.

Gerlach J, Peacock L (1995). New antipsychotics: the present status. *Int Clin Psychopharmacol* **10**(suppl 3):39–48.

Gessa G, Melis M, Muntoni A, Diana M (1998). Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. *Eur J Pharmacol* **341**:39–43.

Gewirtz GR, Gorman JM, Volavka J, Macaluso J, Gribkoff G, Taylor DP, Borison R (1994). BMY 14802, a sigma receptor ligand for the treatment of schizophrenia. *Neuropsychopharmacology* **10**:37–40.

Goff DC, Tsai G, Manoach D, Coyle JT (1995). Dose finding trial of D-cycloserine added to neuroleptics for negative symptoms of schizophrenia. *Am J Psychiatry* **152**:1213–1215.

Grant S, Fitton A (1994). Risperidone. A review of its pharmacology and therapeutic potential in the treatment of schizophrenia. *Drugs* **48**(2):253–273.

Grauer SM, Marquis KL (1999). Intracerebral administration of metabotropic glutamate receptor agonists disrupts prepulse inhibition of acoustic startle in Sprague-Dawley rats. *Psychopharmacology* **141**:405–412.

Grunder G, Muller MJ, Andreas J, Heydari N, Wetzel H, Schlosser R, Schlegel S, Nickel O, Eissner D, Benkert O (1999a). Occupancy of striatal D(2)-like dopamine receptors after treatment with the sigma ligand EMD 57445, a putative atypical antipsychotic. *Psychopharmacology* **146**:81–86.

Grunder G, Wetzel H, Schlosser R, Anghelescu I, Hillert A, Lange K, Hiemke C, Benkert O (1999b). Neuroendocrine response to antipsychotics: effects of drug type and gender. *Biol Psychiatry* **45**:89–97.

Gully D, Canton M, Boigegrain R, Jeanjean F, Molimard JC, Poncelet M, Gueudet C, Heaulme M, Leyris R, Brouard A, Pelaprat D, Labbe-Jullié C, Mazella J, Soubrié P, Maffrand JP, Rostene W, Kitabgi P, Le Fur G (1993). Biochemical and pharmacological profile of a potent and selective nonpeptide antagonist of the neurotensin receptor. *Proc Natl Acad Sci USA* **90**:65–69.

Gully D, Labeeuw B, Boigegrain R, Oury-Donat F, Bachy A, Poncelet M, Steinberg R, Suaud-Chagny MF, Santucci V, Vita N, Pecceu F, Labbé-Jullié C, Kitabgi P, Soubrié P, Le Fur G, Maffrand JP (1997). Biochemical and pharmacological activities of SR 142948A, a new potent neurotensin receptor antagonist. *J Pharmacol Exp Ther* **280**:802–812.

Guyon A, Assouly-Besse F, Biala G, Puech AJ, Thiébot MH (1993). Potentiation by low doses of selected neuroleptics of food-induced conditioned place preference in rats. *Psychopharmacology* **110**:460–466.

Haadsma-Svensson SR, Svensson KA (1998). PNU-99194A: a preferential dopamine D_3 receptor antagonist. *CNS Drug Rev* **4**:42–53.

Hadley MS (1996). D_4 receptors and their antagonists. *Medicinal Res Rev* **16**:507–526.

Hertel P, Fagerquist MV, Svensson TH (1999). Enhanced cortical dopamine output and antipsychotic-like effects of raclopride by α_2 adrenoceptor blockade. *Science* **286**:105–107.

Huber MT, Gotthardt U, Schreiber W, Kreig JC (1999). Efficacy and safety of the sigma receptor ligand EMD 57445 (panamesine) in patients with schizophrenia: an open clinical trial. *Pharmacopsychiatry* **32**:68–72.

Ichikawa J, Meltzer HY (1999). R(+)-8-OH-DPAT, a serotonin1A receptor agonist, potentiated S(−)-sulpiride-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens but not striatum. *J Pharmacol Exp Ther* **291**:1227–1232.

Ishiguro H, Ohtsuki T, Tour M, Itokawa M, Aoki J, Shibuya H, Kurumaji A, Okubo Y, Iwawaki A, Ota K, Shimizu H, Hamaguchi H, Arinami T (1998). Association between polymorphisms in the type 1 sigma receptor gene and schizophrenia. *Neurosci Lett* **257**:45–48.

Javitt DC, Zylberman I, Zukin S, Heresco-Levy U, Lindenmayer J (1994). Amelioration of negative symptoms of schizophrenia by glycine. *Am J Psychiatry* **151**:1234–1236.

Jeanjean AP, Laterre EC, Maloteaux JM (1997). Neuroleptic

binding to sigma receptors: possible involvement in neuroleptic-induced acute dystonia. *Biol Psychiatry* **41**:1010–1019.

Jentsch JD, Redmond DE, Elsworth JD, Taylor JR, Youngren KD, Roth RH (1997). Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine. *Science* **277**:95–955.

Jentsch JD, Taylor JR, Redmond Jr DE, Elsworth JD, Youngren KD, Roth RH (1999). Dopamine D4 receptor antagonist reversal of subchronic phencyclidine-induced object retrieval/detour deficits in monkeys. *Psychopharmacology* **142**:78–84.

Johnson SA, Luu NT, Herbst TA, Knapp R, Lutz D, Arai A, Rogers GA, Lynch G (1999). Synergistic interactions between ampakines and antipsychotic drugs. *J Pharmacol Exp Ther* **289**:392–397.

Josselyn SA, Miller R, Beninger RJ (1997). Behavioral effects of clozapine and dopamine receptor subtypes. *Neurosci Biobehav Rev* **21**:531–558.

Joyce JN (1993). The dopamine hypothesis of schizophrenia: limbic interactions with serotonin and norepinephrine. *Psychopharmacology* **112**:516–534.

Kane J, Honigfield G, Singer J, Meltzer H (1988). The clozaril collaborative study group. Clozapine for the treatment-resistant schizophrenic. *Arch Gen Psychiatry* **45**:789–796.

Kapur S (1996). 5-HT_2 antagonism and EPS benefits: is there a causal connection? *Psychopharmacology* **124**:35–39.

Kapur S, Remington G (1996). Serotonin-dopamine interaction and its relevance to schizophrenia. *Am J Psychiatry* **153**(4):466–476.

Kapur S, Zipursky RB, Remington G (1999). Clinical and theoretical implications of 5-HT_2 and D_2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. *Am J Psychiatry* **156**:286–293.

Karle J, Clemmensen L, Hansen L, Anderson M, Anderson J, Fensbo C, Sloth-Nielsen M, Skrumsager B, Lublin H, Gerlach J (1995). NNC 01-0687, a selective dopamine D_1 receptor antagonist, in the treatment of schizophrenia. *Psychopharmacology* **121**:328–330.

Karlsson P, Smith L, Farde L, Härnryd C, Sedvall G, Wiesel FA (1995). Lack of apparent antipsychotic effect of the D_1-dopamine receptor antagonist SCH39166 in acutely ill schizophrenic patients. *Psychopharmacology* **121**:309–316.

Kasckow J, Nemeroff CB (1991). The neurobiology of neurotensin: focus on neurotensin-dopamine interactions. *Regul Pept* **36**:153–164.

Kehne JH, Baron BM, Carr AA, Chaney SF, Elands J, Feldman DH, Frank RA, Van Giersbergen PLM, McCloskey TC, Johnson MP, McCarty DR, Poirot M, Senyah Y, Siegel BW, Widmaier C (1996). Preclinical characterization of the potential of the putative atypical antipsychotic MDL 100,907 as a potent 5HT_{2A} antagonist with a favorable CNS safety profile. *J Pharmacol Exp Ther* **277**:968–981.

Kikuchi T, Tottori K, Uwahodo Y, Hirose T, Miwa T, Oshiro Y, Morita S (1995). 7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butyloxy}-3;4-dihydro-2(1H)-quinolone (OPC-14597), a new putative antipsychotic drug with both presynaptic dopamine autoreceptor agonist activity and postsynaptic D_2 receptor antagonist activity. *J Pharmacol Exp Ther* **274**:329–336.

Kinkead B, Binder EB, Nemeroff CB (1999). Does neurotensin mediate the effects of antipsychotic drugs? *Biol Psychiatry* **46**:340–351.

Kinon BJ, Lieberman JA (1996). Mechanisms of action of atypical antipsychotic drugs: a critical analysis. *Psychopharmacology* **124**:2–34.

Kramer MS, Last B, Getson A, Reines SA (1997). The effects of a selective D_4 dopamine receptor antagonist (L-745,870) in acutely psychotic patients with schizophrenia. *Arch Gen Psychiatry* **54**:567–572.

Kreiss DS, Bergstrom DA, Gonzalez AM, Huang KX, Sibley DR, Walters JR (1995). Dopamine receptor agonist potencies for inhibition of cell firing correlate with dopamine D_3 receptor binding affinities. *Eur J Pharmacol* **277**:209–214.

Küfferle B, Tauscher J, Asenbaum S, Vesely C, Podreka I, Brücke T, Kasper S (1997). IBZM SPECT imaging of striatal dopamine-2 receptors in psychotic patients treated with the novel antipsychotic substance quetiapine in comparison to clozapine and haloperidol. *Psychopharmacology* **133**:323–328.

Langer SZ, Lehmann J (1988). Presynaptic receptors on catecholamine neurons. In: *Catecholamines I*. Trendelenburg U, Weiner N (editors). Berlin: Springer-Verlag, pp. 419–507.

Larson J, Quach CN, LeDuc BQ, Nguyen A, Rogers GA, Lynch G (1996). Effects of an AMPA receptor modulator on methamphetamine-induced hyperactivity in rats. *Brain Res* **738**:353–356.

Laruelle M, Abi-Dargham A, Gil R, Kegeles L, Innis R (1999). Increased dopamine transmission in schizophrenia: relationship to illness phases. *Biol Psychiatry* **46**:56–72.

Lawler CP, Prioleau C, Lewis MM, Mak C, Jiang D, Schetz JA, Gonzalez AM, Sibley DR, Mialman RB (1999). Interactions of the novel antipsychotic oripiprazole (OPC-14597) with dopamine and serotonin receptor subtypes. *Neuropsychopharmacology* **20**:612–627.

Levant B (1997). The D_3 dopamine receptor: neurobiology and potential clinical relevance. *Pharmacol Rev* **49**:231–252.

Leweke FM, Giuffrida A, Wurster U, Emrich HM, Piomelli D (1999). Elevated endogenous cannabinoids in schizophrenia. *Neuroreport* **10**:1665–1669.

Leysen JE, Janssen PMF, Schotte A, Luyten WHML, Megens AAHP (1993). Interaction of antipsychotic drugs with neurotransmitter receptor sites in vitro and in vivo in relation to pharmacological and clinical effects: role of $5HT_2$ receptors. *Psychopharmacology* **112**:540–554.

Liang X, Wang RY (1998). Biphasic modulatory action of the selective sigma receptor ligand SR 31742A on N-methyl-D-aspartate-induced neuronal responses in the frontal cortex. *Brain Res* **807**:208–213.

Liegeois JF, Bonaventure P, Delarge J, Damas J (1995). Antipsychotics and neuropeptides: the atypical profile of CI-943 and its relationship to neurotensin. *Neurosci Biobehav Rev* **19**:519–531.

Litmat RE, Hong WW, Weissman EM, Su TP, Potter WZ, Pickar D (1993). Idazoxan, an alpha Z agonist, augments fluphenazine in schizophrenic patients: a pilot study. *J Clin Psychopharmacol* **13**:264–267.

Martin WR, Eades CG, Thompson JA, Huppler RE, Gilbert PE (1976). The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J Pharmacol Exp Ther* **197**:517–532.

McAllister KH, Rey B (1999). Clozapine reversal of the deficits in coordinated movement induced by D2 receptor blockade does not depend upon antagonism of alpha2 adrenoceptors. *Naunyn Schmiedeberg's Arch Pharmacol* **360**:603–608.

Meltzer HY (1996). Preclinical pharmacology of atypical antipsychotic drugs: a selective review. *Br J Psychiatry* **168**(suppl 29):23–31.

Meltzer HY (1999). The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology* **21**:1065–1155.

Meltzer HY, Matsubara S, Lee JC (1989). Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin$_2$ pKi values. *J Pharmacol Exp Ther* **251**:238–246.

Millan MJ, Peglion JL, Vian J, Rivet JM, Brocco M, Gobert A, Newman-Tancredi A, Dacquet C, Bervoets K, Girardon S, Jacques V, Chaput C, Audinot V (1995). Functional correlates of dopamine D_3 receptor activation in the rat in vivo and their modulation by the selective antagonist, (+)-S 14297: I. Activation of postsynaptic D_3 receptors mediates hypothermia, whereas blockade of D_2 receptors elicits prolactin secretion and catalepsy. *J Pharmacol Exp Ther* **275**:885–898.

Millan MJ, Gressier H, Brocco M (1997). The dopamine D3 receptor antagonist, (+)-S 14297, blocks the cataleptic properties of haloperidol in rats. *Eur J Pharmacol* **321**:R7–R9.

Millan MJ, Gobert A, Newman-Tancredi A, Audinot V, Lejeune F, Rivet JM, Cussac D, Nicolas JP, Muller O, Lavielle G (1998). S16924 ((R)-2-[1-[2-(2,3-dihydro-benzo[1,4]dioxin-5-γloxy)-ethyl]-pyrrolidin-3yl]-1-(4-fluoro-phenyl)-ethanone) a novel, potential antipsychotic with marked serotonin (5HT) 1A agonist properties: I. Receptorial and neurochemical profile in comparison with clozapine and haloperidol. *J Pharmacol Exp Ther* **286**:1341–1355.

Minabe Y, Matsuno K, Ashby CR (1999). Acute and chronic administration of the selective sigma1 receptor agonist SA4503 significantly alters the activity of midbrain dopamine neurons in rats: an in vivo electrophysiological study. *Synapse* **33**:129–140.

Modell S, Naber D, Holzbach R (1996). Efficacy and safety of an opiate sigma-receptor antagonist (SL82.0715) in schizophrenia patients with negative symptoms: an open dose-range study. *Pharmacopsychiatry* **29**:63–66.

Montgomery AM, Grottick AJ, Herberg LJ (1999) Rapid recovery of self-stimulation responding from depression by clozapine is prevented by the alpha2-adreno-receptor agonist, clonidine. *Behav Pharmacol* **10**:475–482.

Murray PJ, Harrison LA, Johnson MR, Robertson GM, Scopes D-IC, Bull DR, Graham EA, Hayes AG, Kilpatrick GJ, Den Dass I, Large C, Sheehan MJ, Stubbs CM, Turpin MP (1995). A novel series of arylpiperazines with high affinity and selectivity for the dopamine D_3 receptor. *Bioorg Med Chem Lett* **5**:219–222.

Nishikawa T, Hashimoto S, Tanii Y, Umino A, Kashiwa A, Kumashiro S, Nishijima K, Oka T, Shirayama Y, Takahashi K (1994). Disturbed neurotransmission via the N-methyl-D-aspartate receptor and schizophrenia. In: *The Biology of Schizophrenia*. Moroji T, Yamamoto K (editors). Amsterdam: Elsevier, pp. 197–207.

Nutt DJ, Lalies MD, Lione LA, Hudson AL (1997). Noradrenergic mechanisms in the prefrontal cortex. *J Psychopharmacol* **11**:163–168.

Okubo Y, Suhara T, Suzuki K, Kobayashi K, Inoue O, Terasaki O, Someya Y, Sassa T, Sudo Y, Matsushima E, Iyo M, Tateno Y, Toru M (1997). Decreased prefrontal dopamine D_1 receptors in schizophrenia revealed by PET. *Nature* **385**:634–636.

Okuyama S, Imagawa Y, Sakagawa T, Nakazato A, Yamaguchi K, Katoh M, Yamada S, Araki H, Otomo S (1994). NE-100, a novel sigma receptor ligand: effect on phencyclidine-induced behaviors in rats, dogs and monkeys. *Life Sci* **55**:PL133–PL138.

Papp M, Wieronska J (2000). Antidepressant-like activity of amisulpride in two animal models of depression. *J Psychopharmacol* in press.

Perrault Gh, Depoortere R, Morel E, Sanger DJ, Scatton B (1997). Psychopharmacological profile of amisulpride: an antipsychotic drug with presynaptic D_2/D_3 dopamine receptor antagonist activity and limbic selectivity. *J Pharmacol Exp Ther* **280**:73–82.

Poncelet M, Santucci V, Paul R, Guendet C, Lavastre S, Guitard J, Steinberg R, Terranova JP, Breliere JC, Soubrie P, Le Fur G (1993). Neuropharmacological profile of a novel and selective ligand of the sigma site: SR 31742A. *Neuropharmacology* **32**:605–615.

Poncelet M, Barnouin M-C, Breliere J-C, Le Fur G, Soubrie P (1999). Blockade of cannabinoid (CB1) receptors by SR 141716 selectively antagonizes drug-induced reinstatement of exploratory behaviour in gerbils. *Psychopharmacology* **144**:144–150.

Prinssen EP, Ellenbroek BA, Cools AR (1994a). Peripheral and central adrenoceptor modulation of the behavioural effects of clozapine in the paw test. *Br J Pharmacol* **112**:769–774.

Prinssen EP, Ellenbroek BA, Cools AR (1994b). Combined antagonism of adrenoceptors and dopamine and 5-HT receptors underlies the atypical profile of clozapine. *Eur J Pharmacol* **262**:167–170.

Prinssen EPM, Kleven MS, Koek W (1996). Effects of dopamine antagonists in a two-way active avoidance procedure in rats: interactions with 8-OH-DPAT, ritanserin, and prazosin. *Psychopharmacology* **128**:191–197.

Rabinowitz T, Frankenburg FR, Centorrino F, Kando J (1996). The effect of clozapine on saliva flow rate: a pilot study. *Biol Psychiatry* **40**:1132–1134.

Rein W, Turjanski S (1997). Clinical update of amisulpride in deficit schizophrenia. *Int Clin Psychopharmacol* **12**(suppl 2):S19–S28.

Remington G, Kapur S (2000). Atypical antipsychotics: are some more atypical than others? *Psychopharmacology* **148**:3–15.

Reynolds GP (1996). The importance of dopamine D_4 receptors in the action and development of antipsychotic drugs. *Drugs* **51**:7–11.

Richardson-Burns SM, Haroutunian V, Davis KL, Watson SJ, Meador-Woodruff JH (2000). Metabotropic glutamate receptor mRNA expression in the schizophrenic thalamus. *Biol Psychiatry* **47**:22–28.

Richelson E (1999). Receptor pharmacology of neuroleptics: relation to clinical effects. *J Clin Psychiat* **60**(suppl 10):5–14.

Rigton GC, Norman MH, Cooper BR, Howard JL, Boncek VM, Faison WL, Nanry KP, Pollard GT (1996). 1192U90 in animal tests that predict antipsychotic efficacy, anxiolysis, and extrapyramidal side effects. *Neuropsychopharmacology* **15**:231–242.

Rinaldi-Carmona M, Congy C, Santucci V, Simlarid J, Gautret B, Neliat G, Labeeuw B, Le Fur G, Soubrie P, Breliere JC (1992). Biochemical and pharmacological properties of SR 46349B, a new potent and selective 5-hydroxytryptamine$_2$ receptor antagonist. *J Pharmacol Exp Ther* **262**:759–767.

Rinaldi-Carmona M, Barth F, Heaulme M, Alonso R, Shire D, Congy C, Soubrie P, Breliere J-C, Le Fur G (1995). Biochemical and pharmacological characteristics of SR141716A, the first potent and selective brain cannabinoid receptor antagonist. *Life Sci* **56**:1941–1947.

Rinaldi-Carmona M, Pialot F, Congy C, Redon E, Barth F, Bachy A, Breliere J-C, Soubrie P, Le Fur G (1996). Characterization and distribution of binding sites for [3H]-SR 141716A, a selective brain (CB1) cannabinoid receptor antagonist, in rodent brain. *Life Sci* **58**:1239–1247.

Rosse RB, Schwartz BL, Leighton MP, Davis RE, Deutsch SI (1990). An open-label trial of milacemide in schizophrenia: an NMDA intervention strategy. *Clin Neuropharmacol* **13**:348–354.

Rostene W, Azzi M, Boudin H, Lepee I, Souaze F, Mendez-Ubach M, Betancz C, Gully D (1997). Use of nonpeptide antagonists to explore the physiological roles of neurotensin. Focus on brain neurotensin/dopamine interactions. *Ann NY Acad Sci* **814**:125–141.

Roth B, Craigo SC, Choudhary MS, Ulue A, Monsma FJ, Shen Y,

Meltzer HY, Sibley DR (1994). Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J Pharmacol Exp Ther* **268**:1401–1410.

Sanchez C, Arnt J, Dragsted N, Hyttel J, Lembol HL, Meier E, Perregaard J, Skarsfeldt T (1991). Neurochemical and in vivo pharmacological profile of sertindole, a limbic-selective neuroleptic compound. *Drug Dev Res* **22**:239–250.

Sanchez-Arroyas R, Guitart X (1999). Electrophysiological effects of E-5842, a sigma1 receptor ligand and potential atypical antipsychotic, on A9 and A10 dopamine neurons. *Eur J Pharmacol* **378**:31–37.

Santucci V, Gueudet C, Steinberg R, Le Fur G, Soubrié P (1997). Involvement of cortical neurotensin in the regulation of rat mesocorticolimbic dopamine neurons: evidence from changes in the number of spontaneously active A10 cells after neurotensin receptor blockade. *Synapse* **28**:370–380.

Sautel F, Griffon N, Sokoloff P, Schwartz JC, Launay C, Simon P, Costentin J, Schoenfelder A, Garrido F, Mann A, Wermuth C (1995). Nafadotride, a potent preferential dopamine D_3 receptor antagonist activates locomotion in rodents. *J Pharmacol Exp Ther* **275**:1239–1246.

Scatton B, Claustre Y, Cudennec A, Oblin A, Perrault G, Sanger DJ, Schoemaker H (1997). Amisulpride: from animal pharmacology to therapeutic action. *Int Clin Psychopharmacol* **12**(suppl 2):S29–S36.

Schmidt CJ, Sorensen SM, Kehne JH, Carr AA, Palfreyman MG (1995). The role of 5-HT2A receptors in antipsychotic activity. *Life Sci* **56**:2209–2222.

Schoemaker H, Claustre Y, Fage D, Rouquier L, Chergui K, Curet O, Oblin A, Gonon F, Carter C, Benavides J, Scatton B (1997a). Neurochemical characteristics of amisulpride, an atypical dopamine D_2/D_3 receptor antagonist with both presynaptic and limbic selectivity. *J Pharmacol Exp Ther* **280**:83–97.

Schoemaker H, Claustre Y, Peny B, Oblin A, Scatton B (1997b). Dopamine D_3 receptor occupancy in vivo measured by radioligand binding. *Soc Neurosci* **23**:abstract 270.11, p. 683.

Schotte A, Janssen PFM, Gommeren W, Luyten WHML, Van Gompel P, Lesage AS, De Loore K, Leysen JE (1996). Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. *Psychopharmacology* **124**:57–73.

Seeger TF, Seymour PA, Schmidt AW, Zorn SH, Schulz DW, Lebel LA, McLean S, Guanowsky V, Howard HR, Lowe JA, Heym J (1995). Ziprasidone (CP-88,059): a new antipsychotic with combined dopamine and serotonin receptor antagonist activity. *J Pharmacol Exp Ther* **275**:101–113.

Seeman P, Kapur S (1997). Clozapine occupies high levels of dopamine D2 receptors. *Life Sci* **60**:PL207–216.

Seeman P, Tallerico T, Corbett R, Van Tol M, Kamboj RK (1997). Role of dopamine D_2, D_4 and serotonin$_{2A}$ receptors in antipsychotic and anticataleptic action. *J Psychopharmacol* **11**:15–17.

Semba J, Watanabe A, Kito S, Toru M (1995). Behavioural and neurochemical effects of OPC-14597, a novel antipsychotic drug, on dopaminergic mechanisms in rat brain. *Neuropharmacology* **34**:785–791.

Snyder SH, Largent BL (1989). Receptor mechanisms in antipsychotic drug action: focus on sigma receptors. *J Neuropsychiatry* **1**:7–15.

Snyder SH, Greenberg D, Yamamura HI (1974). Antischizophrenic drugs: affinity for muscarinic cholinergic receptor sites in the brain predicts extrapyramidal effects. *J Psychiatr Res* **11**:91–95.

Sokoloff P, Giros B, Martres MP, Bouthenot ML, Schwartz JC (1990). Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. *Nature* **347**:146–151.

Sorensen SM, Kehne JH, Fadayel GM, Humphreys TM, Ketteler HJ, Sullivan CK, Taylor VL, Schmidt CJ (1993). Characterization of the 5-HT_2 receptor antagonist MDL100907 as a putative atypical antipsychotic: behavioral, electrophysiological and neurochemical studies. *J Pharmacol Exp Ther* **266**:684–691.

Sprouse JS, Reynolds LS, Braselton JP, Rollema H, Zorn SH (1999). Comparison of the novel antipsychotic ziprasidone with clozapine and olanzapine: inhibition of dorsal raphe cell firing and the role of $5HT_{1A}$ receptor activation. *Neuropsychopharmacology* **21**:622–631.

Strauss WH, Klieser E (1991). Psychotropic effects of ritanserin, a selective S2 antagonist: an open study. *Eur Neuropsychopharmacol* **1**:101–105.

Takahashi S, Sonehara K, Takagi K, Miwa T, Horikomi K, Mita N, Nagase H, Iczuka K, Sakai K (1999). Pharmacological profile of MS-377, a novel antipsychotic agent with selective affinity for sigma receptors. *Psychopharmacology* **145**:295–302.

Tallman JF (1998). NGD 94-1: a specific dopamine-4-receptor antagonist. *Adv Pharmacol* **42**:490–492.

Tam SW, Cook L (1984). Six opiates and certain antipsychotic drugs mutually inhibit (+)-[^{3}H]SKF 10,047 and [^{3}H]haloperidol binding in guinea pig brain membranes. *Proc Natl Acad Sci USA* **81**:5618–5621.

Thiebot MH, Martin P (1991). Effects of benzodiazepines, 5-HT_{1A} agonists and 5-HT_3 antagonists in animal models sensitive to antidepressant drugs. In: *5-HT_{1A} Agonists, 5-HT_3 Antagonists and Benzodiazepines: Their Comparative Behavioural Pharmacology*. Rodgers RJ, Cooper SJ (editors). Chichester: Wiley, pp. 159–194.

Toru M, Miura S, Kudo Y (1994). Clinical experience of OPC-14597, a dopamine autoreceptor agonist in schizophrenic patients. *Neuropsychopharmacology* **10**(suppl):122S.

Truffinet P, Tamminga CA, Fabre LF, Meltzer HY, Riviere ME, Papillon-Downey C (1999). Placebo-controlled study of the $D_4/5HT_{2A}$ antagonist fananserin in the treatment of schizophrenia. *Am J Psychiatry* **156**:419–425.

Wadenberg ML (1996). Serotonergic mechanisms in neuroleptic-induced catalepsy in the rat. *Neurosci Biobehav Rev* **20**:325–339.

Wadenberg ML, Ahlenius S (1991). Antipsychotic-like profile of combined treatment with raclopride and 8-OH-DPAT in the rat: enhancement of antipsychotic-like effects without catalepsy. *J Neural Transm* **83**:43–53.

Walker JM, Matsumato RR, Bowen WD, Gans DL, Jones KD, Walker FO (1988). Evidence for a role of haloperidol-sensitive sigma-'opiate' receptors in the motor side effects of antipsychotic drugs. *Neurology* **38**:961–965.

Walker JM, Bowen WD, Walker FO, Matsumoto RR, de Costa BR, Rice KC (1990). Sigma receptors: biology and function. *Pharmacol Rev* **42**:355–402.

Wiesel FA, Nordstrom AL, Farde L, Eriksson B (1994). An open clinical and biochemical study of ritanserin in acute patients with schizophrenia. *Psychopharmacology* **114**:31–38.

Willner P (1997). The dopamine hypothesis of schizophrenia: current status, future prospects. *Int Clin Psychopharmacol* **12**:297–308.

Zhang W, Bymaster FP (1999). The in vivo effects of olanzapine and other antipsychotic agents on receptor occupancy and antagonism of dopamine D1, D2, D3, 5HT2A and muscarinic receptors. *Psychopharmacology* **141**:267–278.

Zorn SH, Jones SB, Ward KM, Liston DR (1994). Clozapine is a potent and selective muscarinic M_4 receptor agonist. *Eur J Pharmacol* **269**:R1–R2.

Dopamine Receptor Pharmacology: Interactions with Serotonin Receptors and Significance for the Aetiology and Treatment of Schizophrenia

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Abstract: The classification of dopamine receptors proposed more than two decades ago remains valid today. Based on biochemical and pharmaceutical properties two main classes of dopamine receptors can be distuinguished: D_1-like (D_1, D_5) and D_2-like (D_2, D_3, and D_4) dopamine receptors. Dopamine receptors belong to the class of G protein-coupled receptors and signal to a wide range of membrane bound and intracellular effectors such as ion channels, secondary messenger systems and enzymes. Although the pharmacological properties of ligands for D_1-like and D_2-like dopamine receptors are quite different, the number of selective ligands for each of the five receptors subtypes is rather small. Many drugs used to treat neurological and neuropsychiatric disorders like Parkinson's disease, restless leg syndrome and schizophrenia have affinities for dopamine receptors. Such medications are not without limitations so the development of novel (selective or aselective) dopamine receptor ligands is of the utmost importance for improved therapeutic approaches for these diseases. In that respect it is also important to understand how dopamine receptor ligands affect receptor signalling processes such as desensitization, receptor heterodimerization and agonist-receptor trafficking, issues which will be discussed in the present review. Furthermore, attention is paid to interactions of dopamine receptors with serotonin receptors since many drugs used to treat above mentioned disorders of the brain also posses affinities for serotonin receptors. Because of the enormity of this area we have tried to focus more specifically on interactions within the prefrontal cortex where it appears that the serotonergic modulation of dopaminergic function might be very relevant to schizophrenia.

Keywords: Drug efficacy, signal transduction pathway, receptor classification, neurological and neuropsychiatric disorders, dopaminergic drugs, schizophrenia, restless legs syndrome, prefrontal cortex.

INTRODUCTION

The catecholamine dopamine (DA) is a predominant neurotransmitter found in the brain, but also has an important role in the periphery, including e.g. regulation of cardiovascular function. In both instances the action of DA occurs through the activation of DA receptors and the present review discusses the recent developments in the pharmacology of central nervous system DA receptors. Several DA receptor ligands are effective drugs in treating neurological and neuropsychiatric disorders such as Parkinson's disease and schizophrenia and disturbances of dopaminergic systems in the central nervous system are of critical importance in the aetiology of these diseases. For this reason the development of novel selective dopaminergic drugs is the focus of many research programs both in academic and pharmaceutical industry laboratories. In addition to discussing the pharmacology of DA in this review we also pay attention to the classification, structure, regulation and signal transduction of DA receptors. The latter two aspects of DA receptor function are discussed in the light of the pharmacology of DA receptors and furthermore how these processes can influence (dopaminergic) drug efficacy. In that respect attention is paid to such processes as receptor desensitization, heterodimerization of receptor complexes and agonist-receptor trafficking. Finally, in a separate section we discuss the interaction of DA receptors with serotonin receptors, as many novel antipsychotic drugs (APDs) (e.g. clozapine) possess mixed actions at dopaminergic and serotonergic sites and combined actions at 5-HT and DA receptors may be very important for efficacy versus a reduced side effect profile.

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DOPAMINE RECEPTORS – CLASSIFICATION, STRUCTURE AND REGULATION

Classification of Dopamine Receptors

Five DA receptor sub-types have been classified to date (D_1 through D_5), which are divided into two subclasses, namely D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) DA receptors [1,2]. The classification of these two groups is based on the biochemical and pharmacological properties of the receptors as originally described [3]. Of the DA receptors the D_2 receptor was the first to be cloned [4] and a little later functional splice variants of this receptor were described (i.e. D_{2L} and D_{2S}) [5-7]. In addition, three polymorphic variants of the D_2 receptor exist, encoding for the amino acid substitutions $Val^{96}Ala$, $Pro^{310}Ser$ and $Ser^{311}Cys$ [8]. Initially, due to the lack of selective ligands for the two splice variants the functional and pharmacological properties of the D_{2L} and D_{2S} variants were difficult to determine. However, recent

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studies using knock-out mice for these two receptors have suggested that each splice variant is involved in different physiological processes. For example, the D_{2S} variant is expressed on somatodendritic areas of dopaminergic midbrain neurons and serves as the DA auto-receptor [9], whereas the D_{2L} receptor variant has been demonstrated to function preferentially post-synaptically and in synergistic interactions with other DA receptors such as the D_1 receptor [10,11]. So in general it is believed that the D_{2S} and D_{2L} splice variants are involved in pre- and postsynaptic dopaminergic neurotransmission, respectively [12].

After the cloning of the D_2 receptor two additional DA receptors belonging to the D_2-like subgroup were described, the D_3 receptor [13] and the D_4 receptor [14]. The D_3 and D_4 receptors are much less abundantly expressed in the brain than the D_2 receptor and their expression patterns seem to be restricted to limbic areas [1,15]. Similar to D_2 receptors, splice variants of the D_3 receptors exist but they encode for non-functional proteins (e.g. the truncated variant D_{3nf}; "nf" stands for nonfunctional) [6,16,17]. However, it has been demonstrated that the D_{3nf} variant can interact with the D_3 DA receptor through heterooligomerization and can influence ligand binding [18]. Polymorphic variants of the D_4 receptor in humans have been described with repeat sequences (up to 10 repeats) of 16 amino acids in the third intracellular loop [19,20].

The D_1 receptor was cloned in 1990 [21-24] and the other member of the D_1-like group, the D_5 receptor was cloned a year later [25-27]. Presently no splice variants have been described for D_1-like receptors. D_1 receptors are highly expressed in the typical DA areas like for instance the neostriatum, the substantia nigra (SN) and nucleus accumbens (NAC), whereas the D_5 receptor has a much more restricted expression pattern (e.g. present in the hippocampus and hypothalamus) [2,15].

Structure of Dopamine Receptors

DA receptors belong to the superfamily of G protein-coupled receptors (GPCR) that possess seven hydrophobic trans-membrane spanning domains (7TM receptors), connected by three extracellular and three intracellular loops and which signal *via* G protein-mediated pathways [28]. Seven subgroups or families of 7TM receptors have been distinguished of which the three most important groups are: family A (or family 1), rodhopsin/β2 adrenergic-like receptors (to which the DA receptors belong); family B (or family 2), glucagon/VIP/calcitonin receptor-like; and family C (or family 3), metabotropic neurotransmitter/calcium receptor [29]. In all DA receptors the extracellular N-terminal stretch and the three extracellular loops have approximately similar numbers of amino acids and a variable number of *N*-glycosylation sites: two for D_1 and D_5 receptors, four for D_2 receptors, three for D_3 receptors and only one for D_4 receptors [30]. The highest degree of sequence similarity can be found in the trans-membrane spanning domains, where the homology between D_1-like and D_2-like receptors amounts to 31% [31]. Between members of the same subfamily, the homology in the transmembrane spanning domains is much higher: D_1 and D_5 receptors display a 78% homology, the D_2 and D_3 receptors a 75% homology, whereas for D_2 and D_4 receptors still a 53% homology exists [30]. The transmembrane spanning domains are thought to play an important role in ligand binding [29,32,33], although the second extracellular loop also seems to be involved [32,34].

Cysteine residues in the first and second extracellular loop contribute to receptor structure stabilization by forming an intra-molecular disulfide bridge [35]. In addition, several highly conserved residues, located mainly in the trans-membrane spanning domains, appear to play an essential role in maintaining structural integrity of the receptor [29]. An important difference between D_1-like and D_2-like DA receptors is the much larger third intracellular loop and the much shorter C-terminal stretch of the D_2-like receptors [30,31]. Like with other GPCRs a highly conserved cysteine residue is present in the C-terminal tail of DA receptors and by palmitoylation this site is likely to be anchored within the membrane and thus forms a fourth intracellular loop [36]. The location of the palmitoylated cysteine residue is at the end of the C-terminal tail of D_2-like receptors, whereas in D_1-like receptors (due to the much longer C-terminal tail) from this residue on, a long stretch of the C-terminus extends into the cytoplasmic compartment [1].

Regulation of Dopamine Receptors and Receptor Interactions

DA receptor-mediated activity can, like that of other GPCRs, be regulated through an array of different processes, for example: homologous and heterologous desensitization, sensitization and endocytosis of expressed receptors [37-39]. An important factor involved in drug efficacy is receptor desensitization [40], an adaptational mechanism that prevents receptor overactivation. Upon ligand-mediated receptor activation a cascade of intracellular events is triggered (see below), including the activation of so-called G protein-coupled receptor kinases (GRKs) that are able to phosphorylate serine and threonine residues on the intracellular loops and the C-terminal tail of the GPCR. After a change in receptor configuration due to ligand-mediated activation the GPCR becomes prone to interact with GRKs. When receptor phosphorylation has occurred, another class of proteins involved in GPCR desensitization, the so-called arrestins, can bind to the (activated) receptor, leading to an uncoupling from the G protein signaling pathway, resulting in receptor desensitization [38,41]. In addition, binding of arrestins to the receptor can lead to endocytosis of the receptor from the neuronal membrane [37,41]. It has also been shown that the phosphorylation of the receptor can lead to receptor desensitization through a feedback mechanism *via* the activated second messenger system, e.g. following phosphorylation by protein kinase A (PKA) and/or protein kinase C (PKC) [38]. Desensitization can occur in response to the activation of the own receptor (homologous or agonist-dependent desensitization) but also to unrelated processes, like the activation of another type of GPCR (heterologous or agonist-independent desensitization).

These (general) desensitization processes have also been described for DA receptors, but differences appear to exist [39]. The majority of information is available for D_1 receptors; phosphorylation of serine and/or threonine residues in the 3rd cytoplasmic loop and the C-terminal tail (by PKA or GRKs) plays a role in receptor desensitization,

endocytosis and trafficking (including the sorting of receptors to intracellular compartments) [42-48]. Moreover, it has been suggested that D_1 receptor phosphorylation results in a conformational change of the 3rd intracellular loop and/or the C-terminal tail, allowing arrestin proteins to interact with the activated 3rd loop [48]. The regulation of the D_2 receptor appears to be more complex than that of the D_1 receptor and several studies indicate that the D_2 receptor undergoes multiple positive and negative regulatory events, which can differ largely depending on the cell and tissue type where the receptor is expressed [39]. GRKs and arrestins also mediate D_2 receptor desensitization and endocytosis [49,50], but also PKC-mediated desensitization has been described and phosphorylation of serine and/or threonine residues in the 3rd intracellular loop may contribute to desensitization [51]. Receptor endocytosis seems to be a more common mechanism of D_2 receptor regulation than D_1, perhaps *via* a variety of protein-protein interactions with the D_2 receptor and it is thought that this may underlie the complexity of D_2 receptor regulation [49-53].

Another important consequence for drug efficacy is receptor dimerization. Over the last few years it has become clear that GPCRs can not only form functional homodimers and heterodimers, but also oligodimers [54]. In addition to its consequence for ligand-receptor interactions, dimerization appears significant for receptor signaling regulation processes such as expression, desensitization, sensitization, endocytosis and trafficking [55-59]. In several studies it has been demonstrated that D_2 receptors are able to form dimers [60-62] and it has even been suggested that D_2 receptors are only present as oligomers [63]. Furthermore, oligomerization may have important implications for the pharmacological properties of the D_2 receptor. For example, D_2 receptor dimerization has consequences for cooperativity of ligand binding [60] and changes in DA concentrations can influence D_2 receptor dimerization and consequently ligand binding [64]. The 4th transmembrane domain plays an important role in the formation of D_2 receptor dimers [65,66]. Evidence for D_3 receptor dimerization exists, but it has also been shown that D_3 receptors can form heteromultimers with a splice variant of the D_3 receptor, the D_{3nf} isoform [18,67,68]. When expressed alone, the D_{3nf} variant does not produce functional receptors [6,16,17] but it was suggested that the heteromerization of D_3 and D_{3nf} receptors affects the pharmacological properties of the D_3 receptor and its cellular location [18,67]. It has also been suggested that D_1 receptors are capable of forming dimers [69,70]. An intriguing concept is that DA receptors can form complexes with other GPCRs and thus affect the pharmacological properties of the receptors [71]. It has not only been demonstrated that DA receptors can heterodimerize with other DA receptors (e.g. D_2/D_3 dimers [72] and D_3/D_{3nf} dimers, see above), but DA receptors can also interact with non-dopaminergic GPCRs. So far, the formation of adenosine A_{2A}/DA D_2 dimers [73], adenosine A_1/ DA D_1 dimers [74] and somatostatin SST_5/DA D_2 dimers [75] have been described (which might be important for the treatment of Parkinson's disease). Furthermore, neurotensin receptor activation affects DA agonist binding to the D_2 receptor [76], although neurotensin-DA receptor interactions can also occur downstream of the receptors [77]. Receptor-receptor interactions have not only important implications for understanding how different GPCRs interact at both cellular and network levels [78,79], but they may also have significant consequences for the understanding and treatment of neurological and neuropsychiatric disorders like Parkinson's disease and schizophrenia [80].

SIGNAL TRANSDUCTION THROUGH DOPAMINE RECEPTORS

In this section we will discuss the different effector systems regulated by DA receptor activation. All of these effector systems are coupled to DA receptors through G proteins and we will first give an overview of what is known about the different G protein coupling mechanisms. Subsequently we will discuss the two main effector systems that are coupled to DA receptors (the adenylyl cyclase system and the phospholipases) in more detail. Finally the ion channel systems affected as end targets by DA receptor-mediated signaling pathways will be discussed.

It has to be considered that many of the studies assessing DA receptor-activated signal transduction pathways have been performed in heterologous expression systems (as is actually the case for GPCR-mediated signaling in general) [81]. This means that interactions of DA receptors with their effector systems through different coupling mechanisms are studied in artificial test systems, from which it is difficult to extrapolate to the *in vivo* situation. Indeed, in many cases it still has to be confirmed that the coupling mechanisms and effects on second messenger systems found in the expression systems also exist in the living nervous system. On the other hand, the use of heterologous expression systems has dramatically increased the knowledge about DA receptor signaling and enhanced the insights in DA receptor pharmacology. Furthermore, such studies have facilitated drug discovery processes.

Interactions with G Proteins

DA receptors use G proteins in several signal transduction pathways. As explained above, GPCRs can also interact with many other proteins, of which GRK's and arrestins are only two examples [82]. For dopaminergic GPCRs the third and second intracellular loops are thought to be the major sites that interact with G proteins, although it is not exactly clear through which receptor sequences the coupling occurs [28,29]. GPCRs couple to heterotrimeric G proteins, which are made up of α, β and γ subunits. Four main classes of G proteins are distinguished: G_s (activates adenylyl cyclase), $G_{i/o}$ (inhibits adenylyl cyclase), $G_{q/11}$ (activates phosholipase C) and $G_{12/13}$ (have divers effects) [83-86]. After agonist binding, the receptor complex interacts with the heterotrimeric G protein complex (which is bound to GDP in its resting state), a process that will release GDP and exchange it for GTP. This in turn will result in the dissociation into a Gα-GTP and a G$\beta\gamma$ subunit, allowing the two G protein parts to interact with a wide variety of intracellular constituents. Slow hydrolysis of the Gα-GTP complex through intrinsic GTP-ase activity leads to a re-association of the Gα-GDP and G$\beta\gamma$ subunits and an uncoupling of the whole G protein complex from the receptor, thus closing the cycle and ending the receptor signaling process. These processes can be influenced by a class of GTPase activating proteins, the so-called regulator

of G protein signaling (RGS) proteins, which shorten the life time of the activated state of the G protein by accelerating their hydrolysis of GTP [87].

It was demonstrated that DA receptors can couple to members of all four classes of G proteins. In general, coupling of D_1-like receptors occurs mainly through $G\alpha_s$ and $G\alpha_{olf}$ (stimulation of adenylyl cyclase) and $G\alpha_q$ (stimulation of phospolipase C) and D_2-like receptors preferently couple to effectors *via* members belonging to the $G\alpha_{i/o}$ class of G proteins [28,88]. In addition, for D_1-like and D_2-like receptors it has been demonstrated that $G\beta\gamma$ subunits are able to activate downstream effectors, albeit that presently much more information on this signaling pathway is available for the D_2-like receptors [88].

With the introduction of the concept of "agonist-receptor trafficking" by Kenakin [89] it became clear that the way that GPCRs interact with G proteins may be dependent on the type of agonist that activates the receptor. This concept postulates that different agonists can induce different receptor states with characteristic preferences for coupling to G proteins. So apparently different effector systems can be activated by different agonists interacting with the same receptor and this can have important consequences for the biochemical and pharmacological properties of the receptor under study [90]. Agonist-receptor trafficking may also play a role in D_2 receptor signaling. For example, it was shown that the coupling of D_{2L} and $D_{2Cys311}$ receptors to G proteins can be influenced when the receptors are activated with structurally different agonists and that this occurs by the ability of the agonists to induce different receptor states [91-93]. Finally, RGS proteins have been demonstrated to play a role as modulators of DA receptor-mediated signalling (e.g. RGS4 and RGS9), indicating that these proteins are able to affect DA receptor efficacy and that they may be possible targets for (new) drugs effective in treating neurological and neuropsychiatric disorders which involve disturbances in DA brain systems [87,94-99].

Coupling of Dopamine Receptors to Second Messenger Systems

Adenylyl Cyclase

In general, DA D_1-like receptors are positively coupled to adenylyl cyclase whereas D_2-like receptors usually inhibit this enzyme [15,88]. Adenylyl cyclase is the enzyme responsible for the production of the second messenger cyclic AMP (cAMP) [15,100], which in turn is an activator of PKA, an enzyme that can phosphorylate a large variety of membrane-bound, cytoplasmic and nuclear proteins and thus has a significant effect on cellular physiology [101]. A much studied PKA substrate that plays a central role in DA receptor-mediated signaling is DARPP-32 (dopamine and cyclic AMP-regulated phosphoprotein, 32 kDa) [102]. When phosphorylated this multifunctional signaling protein (that is abundantly expressed in the neostriatum) is a potent inhibitor of protein phosphatase-1, which is a major regulator of the phosphorylation state of many other downstream effectors like neurotransmitter receptors and ion channels. In addition, the latter effectors can also be directly phosphorylated by PKA. For instance, DA D_1 receptor-mediated phosphorylation by PKA of sites on the α-subunit of voltage-dependent Na^+ channels reduces the Na^+ current amplitude [103]. Finally, *via* effects on PKA DA receptors are able to regulate the expression of transcription factors such as cAMP response element-binding protein, demonstrating that DA receptor signaling influences gene expression processes and can consequently shape long term processes such as memory [104-106].

Although the effects of D_1-like and D_2-like receptor activation on adenylyl cyclase are in opposition, in certain cases it has been demonstrated that D_2-like receptors can activate adenylyl cyclase type II. This activation (by D_2 and D_4 receptors, but not by D_3) is mediated by $G\beta\gamma$ subunits, but occurs only when other activators of adenylyl cyclase (e.g. $G\alpha_s$ or PKC) are present [100,107]. Possibly such a synergistic action underlies the cooperative effects of D_1 and D_2 receptor activation on the firing activity observed in nucleus accumbens (NAC) neurons [108].

Phospholipases

DA receptors can convey their signaling through different types of phospholipases [88]. In many cases this concerns phospholipase C (PLC), phospholipase D (PLD) and phospholipase A_2 (PLA_2). Phospholipases are enzymes which catalyze the hydrolysis of phospholipids [109].

PLC hydrolyzes the membrane-bound phospholipid phosphatidylinositol 4,5-biphosphate, resulting in the two intracellular messengers diacylglycerol (which activates PKC) and inositol 1,4,5-triphosphate (which releases Ca^{2+} from intracellular stores) [110]. Evidence has been provided that DA D_1 receptors stimulate PLC through a $G\alpha_q$-mediated process [111-113], whereas other studies suggest that a different D_1-like receptor is involved in this signaling pathway [114,115]. Furthermore, it has been shown that D_5 receptors can inhibit PLC activity, involving $\beta\gamma$ subunits of the G_z protein [116-119]. Like for D_1 receptors also for D_2-like receptors a role in PLC activation has been attributed [120], but more recent studies show that cooperativity of D_1 and D_2 receptors may be important for PLC-mediated signaling [121,122] which may help explain effects seen in *in vivo* systems.

PLD catalyses the hydrolysis of phosphatidylcholine to produce choline and the signaling lipid phosphatidic acid, which in turn can be further metabolized to diacylglycerol and lysophosphatidic acid. Many GPCRs interact with PLD, which can also be activated by PKC and members of the low-molecular weight G protein families Rho and ADP-ribosylation factor [123]. DA receptors are also capable of regulating PLD activity. It has been demonstrated that D_2-like receptors (D_{2S} and D_3) activate PLD through a mechanism that does not involve PKC and G_i/G_o proteins, but is dependent on Rho A [124-126]. Less information is available on interactions of D_1-like receptors with PLD, but recently it was reported that D_5 receptors inhibit PLD expression and activity [127].

PLA_2 is capable of hydrolyzing phospholipids, a reaction that can yield arachidonic acid, the precurser of eicosanoids (which include the prostaglandins) and leukotrines. It is thought that PLA_2 activity not only plays a fundamental role in neuronal injury and inflammatory processes in the brain, but also in processes like nerve regeneration and neuronal cell growth [128], so a role in plasticity and recovery is

suggested. For more than a decade it has been known that DA receptors are involved in the regulation of the release of arachidonic acid evoked by Ca^{2+} [129]. In particular, D_2-like receptors (D_2 and D_4) seem to activate PLA_2 activity [130-132], albeit the D_3 receptor has no effect or inhibits PLA_2 [133]. The exact mechanism underlying the modulation of arachidonic acid release is still unclear, but it is thought that PKC and $G_i\alpha$ and $G\beta\gamma$ subunits may be important players in the regulation of DA receptor-mediated PLA_2 activity [134,135]. It is believed that through D_2-like receptor mediated activation of the arachidonic acid pathway, feedback processes affecting dopaminergic neurotransmission are impacted (e.g. DA re-uptake [136]). No clear evidence has been presented that D_1-like receptors affect PLA_2 activity but early studies suggested that D_1 receptors may facilitate the D_2 receptor-mediated stimulation of arachidonic acid release [129].

Other Effector Systems

Adenylyl cyclase and phospholipases are the most important second messenger systems that are coupled to DA receptors. However, several other effector systems are also controlled by DA receptor activation. In this section we will briefly discuss three such systems: the mitogen-activated protein (MAP) kinases (or extracellular signal-regulated kinases), Na^+/H^+ exchangers and Na^+-K^+-ATP-ase. For more extensive overviews see Missale et al. (1998) [1] and Neve et al. (2004) [88].

Neuronal MAP kinases comprise a family of signaling cascades involved in the regulation of cell division and differentiation and many GPCRs (including DA receptors) are capable of affecting the activity of this cascade [137]. For almost all DA receptors (D_1- and D_2-like receptors) an interaction with MAP kinases has been demonstrated, in most cases involving an activation of the cascade [88].

Intracellular pH, cell volume and transcellular Na^+ absorption are controlled by Na^+/H^+ exchangers, a family of membrane proteins that can be regulated by many GPCRs [138]. In general, D_2-like receptors activate Na^+/H^+ exchangers (but not in all instances), whereas D_1-like receptors seem to regulate Na^+/H^+ exchanger activity in an inhibitory way [1,88].

Na^+-K^+-ATPase is the enzyme responsible for the active exchange of Na^+ and K^+ against their gradients and is thus responsible for maintaining the electrochemical gradient underlying cellular excitability. Furthermore, it is involved in driving the transport of fluid and solutes across the membranes of epithelial cells. It seems that only D_1-like receptors influence Na^+-K^+-ATPase activity (in an inhibitory way), but it has been suggested that in some cases a simultaneous D_2-like receptor activation is necessary for obtaining a maximal effect [1,88].

Coupling to Ion Channels

Since DA receptors couple to many different signalling pathways, it is not surprising that they exert their effect on cell excitability through several ion channels. Among these K^+, Ca^{2+} and Na^+ channels play a prominent role and the direct consequence of this plethora of possibilities is that it is hardly possible to generalize the net effect of DA activation on cell excitability.

Potassium Channels

The DA D_2 receptor is a strong activator of the so-called GIRK (G protein-coupled inward rectifying K^+) channel *via* the $G\beta\gamma$ pathway [139]. As originally demonstrated in the substantia nigra (SN) [140], D_2 receptor activation results in hyperpolarization of the membrane and reduction of the firing rate. This mechanism of action seems to be present in striatal and many other neuron types that contain the D_2 receptor [141]. The D_3 receptor also couples to a GIRK channel and operates approximately through the same mechanism as was demonstrated in rat SN, which expresses predominantly the GIRK2 variant of the GIRK channel [142]. There are indications that in rat SN, the D_2 receptor is much more effective than the D_3 receptor [143]. Despite this rather consistent finding the D_2 receptor can also activate other K^+ currents: in medium spiny neurons the D_2 receptor activates a fast activating, slowly inactivating K^+ current (I_D), but this current is not activated in cortical pyramidal neurons [144]. It is well established that D_2 receptor-initiated GIRK current activation results in a reduced excitability in all neurons.

In contrast with this mechanism, D_1 receptor activation has in general the opposite effect: it reduces voltage-dependent potassium currents mostly through intervention *via* the PKA or DARPP-32 signalling pathway. The reduced K^+ conductance allows the neuron to depolarize and enhances excitability and cell firing. As quite a large collection of K^+ channels can be modulated, the dynamics of the modulation can vary quite dramatically between brain regions. At least two main classes of voltage dependent K^+ channels can be distinguished [145-147]: the fast inactivating (< 20 ms) transient K^+ currents of the A-type (I_A) and the non- or very slowly inactivating (>200 ms) persistent K^+ currents (mainly I_K and I_D). Both classes seem to be modulated by activation of the D_1 receptor, but several signal transduction routes have been implicated. In the NAC, cAMP/PKA was involved in down regulating I_A and so increasing the firing rate in the neurons. However, this study also concluded that a co-regulation of PKA through adenylyl cyclase by D_1 receptors (*via* G_α) and D_2 receptors (*via* $G_{\beta\gamma}$) played a role in regulating the activity of I_A [108]. There are more examples where co-activation of more than one class of DA receptors plays an important role in the regulation of the same target [107,148]. The functional outcome of these modulations becomes even more difficult to predict when specific neurons in a circuit are modulated. One example was reported in the prefrontal cortex (PFC) where PKA-mediated down regulation of K^+-current in GABAergic interneurons through D_1/D_5 receptors, and not through D_2/D_4 receptors, increased firing of these interneurons, so enhancing the GABA release and resulting in an enhanced inhibition of the principal cells [149]. Modulation of this fast transient current in particular affects the repolarization of the action potential and therewith the frequency and pattern of action potential firing. When persistent or slow-inactivating K^+ currents like I_K or I_D are modulated there will be a direct effect on membrane potential and on basic excitability. Examples of this mechanism were described in PFC layer V pyramidal neurons, where D_1, and not D_2 receptors modulate the I_D current, which directly attenuated resting membrane potential and cellular excitability [150]. The complexity of

the DA system is illustrated by the fact that similar neurons from the mPFC showed lowering of spike firing threshold by suppression of a non-inactivating inward rectifying K^+ (IRK) current by D_1 receptor stimulation as well as by D_2-receptor stimulation despite the fact that they exert opposite effects on PKA [151]. Using patch clamp analysis it could be shown that D_1 receptors exert their suppression by direct activation *via* a cAMP route, while D_2 receptors induce suppression of the IRK channel by dephosphorylation [151]. However, D_1 receptor activation was also shown to strongly modulate I_D (in contrast to I_A or I_K) in these neurons. D_2 receptor activation did not have an effect on any of these K^+ currents [152].

Realizing that there are many exceptions, we can summarize the role of dopamine activated K^+ channels in that D_1 receptor activation leads to depolarization and enhanced excitability, while D_2 receptor activation results in hyperpolarization and reduced excitability.

Calcium Channels

The second important class of ion channels that is modulated by DA are the voltage-gated Ca^{2+} channels. Although the molecular variety is smaller than that of the K^+ channels, this does not mean that the possibilities for modulation are less extensive. The reason is that the Ca^{2+} channels, besides their electrical function in the membrane also function as an entry pathway for Ca^{2+} ions into the cell, which results in a large variety of indirect, but highly relevant forms of modulation. Details of the role of intracellular calcium are outside the scope of this review (see e.g. [153]). D_2 receptor activation stimulates PKC, PA_2 and PLD (see paragraphs above). The influx of Ca^{2+}-ions leads to Ca^{2+}-induced inactivation of the L-type current, while the activation of the second messenger systems often involves intracellular Ca^{2+} mobilization with indirect influence on Ca^{2+} currents.

Like the K^+ channels, voltage-gated Ca^{2+} channels can also be divided on the basis of their kinetics into channels that mediate transient inactivating current (N- and T-type) and channels that mediate more persistent current (L- and P/Q-type) (for a review of ion channels see Hille, 2001 [154]). Different Ca^{2+} channel types are often present in the same cell, but they can be differentially localized over the cell compartments, like soma and dendrites. In addition, a highly relevant functional distinction is made between low-voltage-activated Ca^{2+} channels (T-type, threshold around –50 mV), which are generally not strongly modulated and high-voltage-activated Ca^{2+} channels (all the other types, threshold for activation around –25 mV). A typical example of the complexity of Ca^{2+} current modulation is found in the neostriatum where D_2 receptor activation in aspiny cholinergic interneurons down regulates the N-type Ca^{2+} current [155,156] probably resulting in a decrease in acetylcholine release. Activation of D_2 receptors on spiny neurons in the same region down regulates the L-type Ca^{2+} current [157] *via* a similar pertussis toxin dependent $G_{\beta\gamma}$ protein and decreases the glutamate release [158], promoting the low activity state of the neurons [159,160]. D_2/D_3 receptor activation also down regulates hormone secretion in other regions like the pituitary [161] or in cultured AtT-20 cells [162].

Activation of the D_1-receptors has an even more differentiated effect: enhancing L-type currents [163,164] but reducing N- and P/Q-type currents [165]. The PKA and DARPP-32 pathways are involved in the D_1 receptor-mediated down-regulation of the N/P/Q-type Ca^{2+} channels; the consequence is a reduction in Ca^{2+} influx, in excitability and in the tendency to generate calcium spikes [165]. The interaction sites on the Ca^{2+} channel (particularly on the $\alpha 1$ subunit) are known [166,167]. In the situation that Ca^{2+} spikes are generated by this modulation, the localization of the Ca^{2+} current also plays an important role. In pyramidal neurons in the somatosensory cortex the generation of Ca^{2+} spikes, and its modulation by D_1 receptors is mainly located in the distal dendrites [168]. However, Ca^{2+} spikes in neurons in the PFC, also initiated by synaptic activity in the distal dendrites, are generated by Ca^{2+} currents in the proximal dendrites [169].

In summary, modulation of the L-type Ca^{2+} current seems to follow the general pattern established for the K^+ current: D_2 receptor activation reduces L-type Ca^{2+} current and reduces excitability, while D_1 receptor activation has the opposite effect. For the other Ca^{2+} current types more complicated schemes have to be drawn.

Sodium Channels

Functionally, the main role of the Na^+ current in nerve cells is to initiate the upstroke of the action potential. Consequently, any modulation of this Na^+ current will be reflected in the excitability of the neuron. Na^+ currents come in even less functional diversity than K^+ channels or Ca^{2+} channels. Best known is the classic fast-activating and fast-inactivating Na^+ current originally described by Hodgkin and Huxley in the giant squid axon (concise review in Hille, 2001 [154]). About ten molecular distinct α-subunits have been cloned so far, which may differ in their pharmacology and their sensitivity to modulation. Without excluding a subtle (pharmacological) role for this considerable subunit variation in the fast Na^+ channel, we will treat the fast Na^+ current in this review as one functional class. In addition to this class a persistent Na^+ current has also been described; it has quite comparable activation characteristics as the fast Na^+ channel, but inactivates at a time scale of seconds instead of the classical few milliseconds [170]. This persistent Na^+ channel has not been molecularly identified and it is therefore debated whether we are dealing with a distinct channel or with a special functional state of the classical Na^+ channel. Functionally, the persistent Na^+ current is difficult to separate from the also often present window current through the fast Na^+ channel: in the voltage range where the inactivation and the activation function overlap, a small residual Na^+ current can be observed. The amplitude of the persistent and the window current is several orders of magnitude smaller than the maximal amplitude of the fast Na^+ current, but the difference in kinetics makes it highly suitable to influence membrane potential at least by several millivolts.

D_1 receptor activation modulates the fast Na^+ channel by phosphorylation through the PKA / DARPP-32 pathways [103,171]. It down regulates the channel, reduces excitability and enhances spike threshold [172,173]. It has been suggested that D_1 receptor-induced phosphorylation of the Na^+ channel is involved in state switching of neostriatal

dopaminergic neurons [174]. Modulation of the persistent Na^+ current could happen along the same pathways, resulting in a reduction of the current amplitude, continuous depolarization and accompanied by a lower excitability [170,175,176]. It seems however, that the outcome of D_1 receptor modulation on the persistent current is much more variable and results are quite different in responses between brain regions, as for example an enhancement of the current has been reported in the PFC [177]. D_2 receptor activation also leads to a variety of changes in the Na^+ channel activity either up or down regulated [177,178]. In neostriatal neurons a decrease in Na^+ current was reported, mediated by $G_{\beta\gamma}$ binding and mechanistically explained by a shift of the inactivation function of the Na^+ current in hyperpolarizing direction [144], an explanation supported by work in cultured cell lines [179].

As most neurons contain a considerable subset of K^+ as well as of Ca^{2+} and Na^+ currents, which cooperate in close harmony in the same voltage range, it is important to always consider the modulation of both current types by D_1- and/or D_2-like receptors when trying to evaluate the net functional outcome of such a modulation in terms of its effect on neuronal firing activity.

Summary

DA receptor signaling appears to be complex and diverse and many transduction pathways and effector systems that are linked to the different DA receptor subtypes can be distinguished. In addition, DA receptor pharmacology is equally complex and processes like receptor desensitization, heterodimerization of receptor complexes and agonist-receptor trafficking may be involved in shaping the pharmacological properties of DA receptors. In the following section we will focus on the pharmacology of DA receptors and discuss this in the light of the aetiology and treatment of neurological and neuropsychiatric disorders like Parkinson's disease, restless legs syndrome (RLS) and schizophrenia. Especially for the latter disorder the interaction of DA receptors with 5-HT receptors will be discussed since this interaction may play a significant role in the disease process and in addition may be of importance for the effectiveness of (new) APDs.

PHARMACOLOGY OF DOPAMINE RECEPTORS

The literature describing the pharmacology of DA receptors is very extensive. The affinities of drugs for DA receptors belonging to the same group (D_1-like or D_2-like) are more or less in the same range, whereas the differences in affinities of drugs for receptors of two different groups may be substantial. The differences in pharmacology of D_1-like and D_2-like receptors as originally described more than two decades ago are still valid [3], yet a limited number of purely selective ligands are available for each of the five DA receptor subtypes. However, there have been significant developments in the last few years. For example, several selective D_3 (e.g. S33084) and D_4 receptor ligands (e.g. PD168077, L741,741, ABT724) are presently available. Table 1 demonstrates an overview of drugs possessing affinities for the DA receptors. In general, D_1-like receptors have a relatively high affinity for benzazepine ligands (e.g. A68930, fenoldapam, SCH23390), whereas the D_2-like receptors have a preference for buthyropherone (e.g. spiperone, haloperidol) and substituted benzamide ligands (e.g. raclopride, remoxipride, sulpiride). As can be seen in Table 1 for several compounds a large variation in K_i values for the same receptor type has been reported (see e.g. iloperidone) and this may depend on whether agonist or antagonist binding has been performed or on the expression of receptors in cell systems or from native tissue.

Disturbances of CNS DA systems are involved in several neurological and neuropsychiatric disorders such as Parkinson's disease, RLS and schizophrenia to name just a few. In line with this, drugs with affinities for DA receptors have proven to be effective in treating these disorders. However, no real dopaminergic "wonder" drugs have been developed to treat these disorders so far. In the case of schizophrenia, for example, the idea emerged during the 1990's that finding a selective D_4 receptor ligand could have APD efficacy since it was found that in schizophrenics D_4 receptor densities were increased and that the atypical APD clozapine had a relatively high affinity for this receptor [20] (Table 1). The finding however that the selective D_4 receptor antagonist L-745,870 was ineffective as APD in humans [222] demonstrates that a complex disorder like e.g. schizophrenia cannot be explained (and therefore be treated) by disturbances at the level of a single receptor.

There are many excellent reviews discussing the therapeutic actions of dopaminergic drugs in Parkinson's disease [216,223,224] and schizophrenia [225-227] (insofar as the DA precursor l-3,4-dihydroxyphenylalanine (L-DOPA) can be used to treat symptoms of the disease but this is often associated with wearing off, 'on-off' symptoms and the development of dyskinesia; therefore, dopamine agonists such as pramipexole and ropinirole are proposed as first-line treatment in order to "save" L-DOPA). However, relatively less attention has been paid to RLS. Therefore we briefly discuss the literature regarding the therapeutic actions of dopaminomimetic drugs in RLS and further we discuss specific interactions of dopaminergic and serotonergic systems and potential implications for psychotic disorders such as schizophrenia.

Restless Legs Syndrome

Restless legs syndrome or Ekbom's syndrome is characterized by the urge to move the legs often accompanied or caused by uncomfortable and unpleasant sensations in the limbs which are worse during restlessness and inactivity, partially relieved by movement and more severe at night or in the evening (for review see [228]) and is a major cause of insomnia. RLS is a generally under-diagnosed disorder with a relative prevalence of 1-15% of the general population [229,230]. The cause of RLS remains unclear. Given the treatment efficacy of dopaminomimetics and links of Parkinson's disease with RLS many have considered a link with DA. Cerebrospinal fluid from RLS patients was analysed and no changes were found in age-corrected contents of the DA metabolite homovanillic acid (HVA) (and neopterin), while the 5-HT metabolite

Table 1. Affinity Values of Dopaminergic Ligands for the Five Dopamine Receptor Subtypes (K_i Values, in nM Unless Stated Otherwise)

	D_1-like		D_2-like		
	D_1	D_5	D_2	D_3	D_4
Genetic Locus	5q-34-35[21]	4p-15.1-15.3[180]	11q-22-23[181,182]	3q-13.1[183]	11p-15.5[14,184,185]
Antagonists					
A437203			348[186]	29[20]	
(+)-AJ76			155[186]	2-6[186]	
Amisulpiride	>10μM[187]		21[187]	2.9[187]	
Amperozide	3.2μM[188]		390-540[188]		384[188] ($D_{4.4}$)
Blonanserin	2.9μM[189]		14.8[189]		
Chlorpromazine	~90[20]	~130[20]	3[20]	4[20]	35[20]
Clozapine	~170[20] 540[190] 85[191] 290[192]	~330[20]	125[191] ~230[20] 160[190] 130[192]	~170[20] 360[190] 240[192]	21[20] 27.9[193] 54[192]
N-desmethyl-clozapine	1.3μM[194]		1.2μM[194]		
DS121			1140[186]	249[186]	
Eticlopride	18μM[195]	19μM[195]	0.03-0.4[195]	0.02[195]	2[195]
FAUC 365			3.6μM[186]	0.5[186]	
Flupentixol, *cis*	0.7-21[195]	0.7-24[195]	0.1-5[195]	0.1-1.1[195]	
Fluphenazine	21-28[195]	14[195]			
Flusprilene	450[190]		1.5[190]	1.1[190]	
GR103691			24[186]	0.4[186]	
GR218,731	<1μM[196]	<1μM[196]	63[196]	1[196]	10μM[196]
Haloperidol	~80[20] 270[190] 25[191] 120[192]	~100[20]	~1 [20,190-192,197]	~7[20] 21[190] 2.5[192]	2.3[20] 1.9[193] 5[191] 3.3[192]
Iloperidone	1060[198] 200[199]	316[199]	21.4[199] 111[198]	79[199]	25[199]
JL18	398[200]		530[200]		21[200]
KCH1110			118.8[186]	1.28[186]	
L741,626	794[196]	631[196]	4[196]	7.2[196]	316[196]
L741,741			>1.7μM[158]	480[158]	2.5[158]
L741,742			>1.7μM[158]	770[158]	3.5[158]
L745,870					0.37[193]
LE 300	1.9[201]		44.7[201]		
Loxapine			5.2[202]		7.8[202]
Melperone			88[202]		420[202]
Molindone			6[202]		2.4μM[202]
Nafadotride			5[186]	0.52-0.88[186]	
Nemonapride			0.06, <1[197]	0.3[20]	0.15[20]
NGB2849	>10μM[203]	>10μM[203]	262[203]	0.9[203]	>5μM[203]
NGB2904	>10μM[203]	>10μM[203]	217[203]	1.4[203]	>5μM[203]
NGD 94-1	<10μM[204]	<10μM[204]	2.2μM[204]	<10μM[204]	3.6[204]
(+)-N-propyl-norapomorphine			275[200]		13-120[200]

(Table 1) contd.....

	D_1-like		D_2-like		
	D_1	D_5	D_2	D_3	D_4
Olanzapine	31[191] 250[190] 52[192]		45[20] 11[191] 17[190] 20[192] 15[197]	4.2[190] 45[192]	27[191] 60[192]
ORG5222	24[190] 54[191]		2[190] 1[191]	4.5[190]	
PD89211					3.7[205]
Perlapine			60[202]		30[202]
Pimozide			0.4-10[195]	0.4-11[195]	43[195]
Pipamperone	2.5μM[190]		93[190]	480[190]	
Raclopride	18μM[20]		1.8[20]	3.5[20]	2.4μM[20]
Remoxipride			~300[20]	~1.6μM[20]	~2.8μM[20]
Risperidone	580[192]		~5[20] 2.2[192] 2[197]	6.7[20] 9.6[192]	7[20] 8.5[192]
9-OH-Risperidone	640[190]		4[190]	7.5[190]	
S14297			297[186]	4.9-15[186]	
S18327	48[206]	158[206]	78[206]	107[206]	6[20]
S33084	501[196]	1.3μM[196]	52-29[186] 32[196]	<1[186,196]	2μM[196]
SB277011A			1μM[186]	10[186]	~3μM[20]
SB414796			398[186]	4[186]	
SCH23390	0.1-0.4[2]	0.1-0.5[2]	270-1100[2]	314-800[2]	~3μM[2]
SCH39166	1.2[339]	2.0[339]	980[339]		5520[339]
Seroquel (Quetiapine)	4240[190] 455[191] 1.3μM[192]		310[190] 160[191] 180[192]	650[190] 320[192]	2.2μM[192]
Sertindole	210[190]		7.4[190]	7.5[190]	
SKF83566	2.7[207]				
SLV313			3[208]	8[208]	4[208]
SLV314			<1[197]		
Spiperone	~350[20]	~3.5μM[20]	0.06[20]	0.6[20]	0.08[20]
SSR181507	>1μM[209]	>1μM[209]	0.84[209] 4[197]	0.18[209]	825[209]
(S)-sulpiride	~4.5μM[20]	77μM[20]	~15[20]	~13[20]	1μM[20]
(R)-sulpiride	~19μM[20]	29μM[20]	~900[20]	~400[20]	970[20]
Thioridazine			0.4[202] 5[197]		1.5[202]
Tiapride	>10μM[210]		110-320[210]	180[210]	>10 μM[210]
Tiospirone			6[197]		
Trifluperazine			0.96[202]		44[202]
U-99194-A			2.3μM[186]	160-223[186]	
(+)UH232			28[186]	3.3-7[186]	
YM-09151-2			0.02-0.09[195]	0.04-0.06[195]	0.04-0.06[195]
Ziprasidone	130[192]		3.1[192] 13[197]	7.2[192]	32[192]
Zotepine	34[211]		9[211] 8.1[212]		

(Table 1) contd.....

	D_1-like		D_2-like		
	D_1	D_5	D_2	D_3	D_4
Agonists					
A369508	>10μM[193]	>10μM[193]	1.8μM[193]	4.1μM[193]	4[193]
A68930	3[213]		776[213]		
A70108	2[213]		813[213]		
A77636	7.4[214]		5.9[214]		
ABT-724	>10μM[215]	>10μM[215]	>10μM[215]	>10μM[215]	63.6[215] ($D_{4.4}$)
(-)-apomorphine	~0.7[20]		~0.7[20]	~32[20]	~4[20]
(+)-apomorphine			~75[20]		15[20]
(+/-)-apomorphine	372[216] 550[217]	15[216]	83[216] 98[217]	26[216]	4[216]
Bromocriptine	63[218] 692[216] 2.4μM[217]	537[216]	6[218] 15[216] 31[217]	372[216]	501[218] 537[216]
Cabergoline	692[216]	22[216]	1[216]	<1[216]	22[216]
CP226229					0.8[193]
Dihydrexidine	6.2[219]		1.5μM[219]	170[219]	
Dinapsoline	5.9[219]		174[219]		
Dopamine	0.9[20]	<0.9[20]	~7[20] 10[215]	~4[20]	~30[20] 56.2[215]
Fenoldapam (SKF82526)	25[217]	11-27[195]	620[217]		
Lisuride	65[216]	8.45[216]	1[216]	<1[216]	10μM[216]
LU 24-040	50[217]		2.3μM[217]		
Naxagolide	80[20]		~1.5[20]		
NN-dipropyl-5,6DTN	360[217]		140[217]		
(-)-NPA	500[217]		9.4[217]		
7-OHDPAT	~5μM[20]		10[20] 92[186]	~1[20] 0.34-2.2[186]	650[20]
Pergolide	0.8[20] 3.39[216] 1μM[217]	33[216]	~0.8[20] 26[216] 26[217]	~1.5[20] 5[216]	59[216]
PD128907			339[186]	1.3-1.9[186]	
PD1608077	4.6μM[193]	4.5μM[193]	1.3μM[193]	2.3μM[193]	11.9[193]
PNU-95666E			53.8[215]		
Pirebedil	>10μM[216]	>10μM[216]	6.8[216]	6.6[216]	6.5[216]
Pramipexole	>5μM[219] >10μM[216]	>10μM[216]	1.7μM[216]	10[216]	129[216]
Quinelorane	>10μM[216,217,219]	>10μM[216]	710[219] >10μM[219] 708[216] 130[217]	5[216]	19[216], 0.7[219]
Quinpirole	1.9μM[20] 7.9μM[218] >10μM[216]	>10μM[216]	4.8[20] 7.9μM[218] 1.5μM[216]	~24[20] 34[216]	~30[20] 200[218] 34[216]
Ropinirole	>10μM[216]	>10μM[216]	933[216]	37[216]	851[216]
Roxindole	468[216]	191[216]	2[216]	1[216]	6[216]
SKF81297	11[217]		3.7μM[217]		
Talipexole	2.5μM[218] >10μM[216]	3.5μM[216]	200[218] 977[216]	68[216]	1.3μM[218] 331[216]

(Table 1) contd.....

	D_1-like		D_2-like		
	D_1	D_5	D_2	D_3	D_4
TL99	2.7μM[216]	78[216]	68[216]	3[216]	58[216]
(+)-3-PPP	43μM[217]		24μM[217]		
Partial Agonists					
Aripiprazole	2μM[220]	2.6μM[220]	3[20] 0.7-3.3[220]	9.7[220]	510[220]
BP897			61[186]	0.92[186]	
(-)-3-PPP	631[218]		501[218]		3.2μM[218]
Sarizotan	6.7μM[221]	634[221]	15[221] 7[155]	17[221]	4[221]
SKF38393	1[20] 85[217] 740[207]	~0.5[20]	~150[20] 48μM[217]	~5μM[20]	~1μM[20]
SKF75670	130[207] 23[217]		1.4μM[207] 4.4μM[217]		
SLV308	32[218]		32[218]		25[218]
Terguride	28[216]	23[216]	1[216] 3[218]	1[216]	8.09[216] 8.6[218]

Relatively high affinity values of compounds (low K_i values, compared with the (high) values of the same compound for other DA receptor subtypes) are indicated by the shaded cells in which these values are listed. Where necessary K_i values were calculated from pK_i's using $pK_i = -\log K_i$. Furthermore, the (human) chromosome positions of the genes encoding for the dopamine receptor subtypes are listed (genetic locus).

5-hydroxyindoleacetic acid and tetrahydrobiopterin were reduced [231]. Positron emission tomography (PET) studies suggest that D_2 receptor binding is reduced in the striatum in L-DOPA or treatment-naïve patients (compared to age-matched controls) [232,233]. DA uptake binding was either slightly reduced or unaffected, respectively [232,233]. In successfully treated patients no changes in the ratio of striatum:frontal cortex D_2 receptors were seen (compared to age-matched controls), leading to the suggestion that positive treatment efficacy may result in normalisation of such measures [234]. Nocturnal (but not day-time) L-DOPA neuroendocrine challenge studies suggest a pronounced inhibition of prolactin release and an increase in growth hormone levels in RLS patients; moreover prolactin plasma levels were significantly correlated to the extent of disease [235], suggesting the link between the disease and dopaminergic D_2 receptor tone. These data suggest that diurnal variation in DA responsiveness could account for the symptomatologies. It has been suggested that diurnal variations in melatonin might modulate DA responsiveness leading to worsening of RLS symptoms [236]. While opioids and benzodiazepines have proved efficacious for the treatment of RLS, dopaminomimetics have become the mainstay of treatment strategies. A variety of DA agonists have been tested in clinical trials e.g. ropinirole, rotigotine (patch), cabergoline, pirebedil, pergolide and pramipexole, and have all demonstrated efficacy for the treatment of the disease [237-242]. Basic research leading to novel treatment strategies for RLS are limited due to the poor availability of animal models. For example, studies in animals have assessed periodic limb movements, the role of iron deficiency or lesions of specific lesions of the A8 and A11 dopaminergic regions, all as potential animal models but they have limited applicability to the clinical situation (for review see [243]). More recently, it has been suggested that lowered dopaminergic tone decreases the spinal monosynaptic stretch reflex in wild-type mice, while in DA D_3 receptor knockout mice this was reversed by the D_3 agonists pergolide and PD128907 and in turn increased by the D_3 antagonist GR103691 [244]. No such effects were seen in homozygote knockout mice suggesting a key role of the D_3 receptor leading to the suggestion that possibly RLS symptoms might be due to changes in D_3 receptor function in the spinal cord [244]. Circadian changes in spinal DA production in D_3 knockout mice support such a concept [245]. However, these latter studies focus on electrophysiological studies *ex vivo* in isolated organs and while it is interesting to speculate that the disease might involve such mechanisms given the putative actions of anti-RLS medications at D_3 receptors (such as ropinirole) further *in vivo* studies will need to be conducted to assess whether changes in this spinal reflex contribute to the disease process.

SEROTONERGIC AND DOPAMINERGIC RECEPTOR INTERACTIONS, RELEVANCE FOR SCHIZOPHRENIA

The hypofrontality model of schizophrenia proposes a hypoactive medial prefrontal circuitry in schizophrenia [246]. Studies have demonstrated that DA levels in the mPFC can be augmented by serotonergic compounds (e.g. [247,248]). A "normalization" of reduced DA function may have a role in decision making, working memory and attentional processing [249] and may also have an additional role in the treatment of negative symptoms of schizophrenia [246]. For example, preclinical studies have demonstrated that 5-HT_{1A} receptor agonism appears to improve attentional processing in the mPFC [250] and the 5-HT_{1A} partial agonist tandospirone improved cognition in schizophrenic patients

[251,252]. Therefore a discussion of serotonergic interactions at the level of the mPFC is warranted not only from a basic science perspective but also for its implications in schizophrenia.

Serotonin and Serotonin Receptors

Following the initial discovery of serotonin (5-hydroxytryptamine; 5-HT) in the intestine [253], serum [254] and brain [255] and the description of the 5-HT_D and 5-HT_M receptor subtypes [256], a wide variety of serotonergic receptors has been shown to exist. To date up to fifteen serotonergic receptor subtypes have been described: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3 (although compelling evidence exists for 5-HT_{3A} and 5-HT_{3B} receptors), 5-HT_4, 5-HT_{5A}, 5-HT_{5B}, 5-HT_6 and 5-HT_7. Additionally, a variety of splice variants have been described for various receptor types (for review see [257]). Many of the serotonin receptors have been implicated in the modulation of the dopaminergic system: including the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_4 and 5-HT_6 receptors and as such may have relevance for schizophrenia. Like dopaminergic receptors a wide variety of selective pharmacological agents have been developed to assess the role of serotonergic receptors and transporters in the brain and these have been adequately reviewed elsewhere (e.g. [257]).

5-HT Efferents and Afferents to Dopaminergic Nuclei

The serotonergic cell body regions were arbitrarily assigned as B1-B9 [258] and while they penetrate essentially all areas of the central nervous system, B1 and B4 project to the spinal cord and brain stem. The rostral serotonergic nuclei of the dorsal raphe nucleus (DRN; B7 and its caudal extension B6), median raphe nucleus (MRN; B8 and B5) and B9 (a lateral extension of the base of the MRN) project to forebrain regions [259-262]. Efferent projections from both the MRN and DRN connect with the SN zona compacta (SNc; A9) and the ventral tegmental area (VTA; A10), whereas the projection areas of the SNc and VTA (the striatum and NAC) receive afferent projections from the DRN while the mPFC has both DRN and MRN afferents [263-269]. Within the VTA, SN, NAC, striatum and mPFC and even the DRN there exists a putative interaction between serotonergic and dopaminergic systems.

Serotonergic Regulation of Dopamine Transmission in the Prefrontal Cortex

Serotonergic afferents to the PFC affect dopaminergic neurotransmission. Early studies examined the effect of local 5-HT application on extracellular DA levels in the PFC and striatum respectively [270]. Local 5-HT application dose-dependently increased extracellular DA levels in both regions, but this effect was more marked in the PFC compared to the striatum. Characterization of the 5-HT receptor subtype responsible for modulating extracellular DA levels has suggested that the 5-HT_{1A}, 5-$HT_{1B/1D}$, 5-HT_{2A}, 5-HT_{2C}, 5-HT_4 and 5-HT_6 receptors may be involved in these actions [270]. However, studies have not yet elucidated any role of the 5-$HT_{1E/1F}$, 5-HT_{2B}, 5-$HT_{5A/5B}$ or 5-HT_7 receptors in modulating frontal dopaminergic function.

5-HT Reuptake Regulation of Dopamine Transmission in the Prefrontal Cortex

A number of studies have examined the influence of 5-HT on dopaminergic neurotransmission by assessing the effects of serotonin specific reuptake inhibitors (SSRIs) on extracellular DA levels in the PFC [271-274]. For example, systemic fluoxetine increased extracellular DA levels in the PFC, while citalopram, fluvoxamine, paroxetine and sertraline failed to elicit this effect when tested at doses that caused elevation of synaptic 5-HT [272]. The degree of selectivity of the SSRIs may well impact on the results observed. Dose-dependent increases in PFC DA levels have been reported by others following systemic fluoxetine but not fluvoxamine administration [275] although local PFC application of fluoxetine and fluvoxamine alone were associated with increased extracellular PFC DA levels. However, controversies in the literature exist; others have demonstrated that systemic administration of paroxetine and citalopram can also increase extracellular PFC DA levels [276,277]. These effects appear to be specific to the PFC as administration of systemic fluoxetine and citalopram alone failed to alter extracellular DA levels in the NAC [277]. Paroxetine and fluoxetine-induced increases in PFC DA levels were reversed by the 5-HT_3 receptor antagonist granisetron [277] but fluoxetine was able to antagonize 5-HT_{2C} receptors [272], suggesting that the increase of extracellular DA levels in the PFC could be independent of SSRI characteristics and/or mediated *via* other 5-HT receptors (either directly or indirectly) [277].

In vivo characterization of the 5-HT releaser 3,4-methylenedioxy-methamphetamine (MDMA)-induced 5-HT release suggested that increases in dopaminergic neurotransmission are observed that are dependent on serotonergic mechanisms [277,278], further supporting a facilitatory role of serotonergic systems on PFC dopaminergic function, although MDMA also releases DA itself.

The interaction of serotonin with dopaminergic systems in the cortex has obvious implications for the treatment of a range of diseases most notably including depression and schizophrenia [273,279].

Combination of SSRIs and APDs on Dopamine Release

Dopamine D_2 receptor antagonists increase synaptic DA levels in the mPFC [280]. Evidence suggests that the SSRI fluvoxamine (at an effective dose to increase 5-HT) did not have an effect on extracellular DA levels in the PFC and striatum. However, when the $D_{2/3}$ receptor antagonists sulpiride or haloperidol were co-administered with fluvoxamine enhanced DA levels in the PFC but not striatum, were seen, in contrast to DA $D_{2/3}$ antagonists alone. This suggests potential atypical-like actions (see later) which could be reduced by 5-HT_{1A} receptor antagonism using WAY100,635 [281]. The systemic combination of the APD quetiapine with fluvoxamine resulted in an increase of extracellular DA levels in the rat dorsal striatum, PFC, NAC and thalamus [282]. Interestingly, quetiapine alone had no effect on extracellular DA and 5-HT levels in the PFC and thalamus, but was associated with an increase in extracellular DA and 5-HT levels in the dorsal striatum and NAC [282].

Nonetheless, quetiapine possesses weak effects at D_2 receptors [283], consistent with low occupancy at the D_2 site but which does coincide with clinical efficacy [284-286] and possesses a PET profile similar to that of clozapine (see [284]).

Many classical and atypical APDs increase mPFC DA but the selective actions in the mPFC (*vs.* the striatum) appears restricted to the atypical agents (for example [287-291] and it is believed that 5-HT_{1A}-5-HT_{2A} receptor modulation plays an important role in these effects.

5-HT_{1A} Receptor Regulation of Dopamine Neurotransmission in the Prefrontal Cortex

The 5-HT_{1A} receptor agonist (±)-8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT) increases basal DA utilization and release in the mPFC [292], while decreasing 5-HT release [293]. Interestingly, 8-OH-DPAT attenuated PFC DA release elicited by mild footshock stress, suggesting that 5-HT_{1A} receptors regulate DA overflow in the PFC under overt and stressful conditions.

Stimulation of 5-HT_{1A} receptors influences DA turnover in extra cortical areas, which may be relevant for schizophrenia: 8-OH-DPAT decreased L-DOPA accumulation in rat striatum following administration of the l-aromatic amino acid decarboxylase inhibitor NSD-1015 [294]. Striatal L-DOPA accumulation induced by amphetamine was attenuated by 8-OH-DPAT. However, both amphetamine and 8-OH-DPAT decreased cortical L-DOPA accumulation but did not influence L-DOPA accumulation in the NAC [294]. 8-OH-DPAT blocked amphetamine-induced increases in extracellular PFC DA and 5-HT levels which were reversed by the 5-HT_{1A} receptor antagonist WAY100,635 [295]. Also, 8-OH-DPAT potentiated the ability of a low (but not high) dose of the D_2-like DA receptor antagonist sulpiride to increase DA release in the NAC but not in the striatum in a 5-HT_{1A} receptor-dependent manner [248]. Taken together, this suggests that the relative tone of the dopaminergic system might be very important with respect to dopamine – serotonergic interactions mediated *via* the 5-HT_{1A} receptor. The 5-HT_{1A} agonists ipsapirone and buspirone dose-dependently increased dialysate DA levels in the rat PFC [296] and acute, but not chronic, systemic buspirone administration increased impulsivity which was blocked by WAY100,635 [297], which may be related to the PFC. Interestingly, the ability of ipsapirone to increase DA release in the rat PFC was antagonized by the partial 5-HT_{1A} agonists NAN-190 and WAY100,135. When given alone NAN-190 also increased dialysate DA levels in the PFC while WAY100,135 alone had no effect. A similar result was seen when the low affinity 5-HT_{1A} agonist mirtazapine was used. Mirtazapine (also an α_2 adrenergic antagonist and 5-HT_{2A} receptor antagonist) dose-dependently increased DA but not 5-HT levels in the PFC [298] which was attenuated in the presence of WAY100,635. Systemic administration of the 5-HT_{1A} agonist flibanserin reduced dialysate PFC NA and 5-HT levels but increased PFC DA levels, all of which were reversed in the presence of WAY100,635 (which by itself had no effect on any of the monoamines tested) [299]. Taken together, these results suggest that 5-HT_{1A} receptor stimulation is associated with increased PFC DA release. The atypical APD aripiprazole, a 5-HT_{1A} receptor partial agonist (and partial DA D_2 agonist), has been tested for its effects on systemic DA levels measured in the PFC and striatum at relatively high doses [300]; acute and chronic systemic administration failed to alter DA release in both the PFC and striatum in contrast to olanzapine which induced a preferentially increase in frontal DA but was associated with increased levels of the DA metabolites DOPAC and HVA in the same brain regions. Chronic administration (21 days) was associated with a marginal reduction in PFC dopamine compared to basal, although a reduction was also seen after vehicle administration and no significance from vehicle was observed. However, in another study [289] low doses of aripiprazole (0.1 and 0.3 mg/kg, but not at higher doses) augmented PFC and hippocampal DA (effects reversed by WAY 100,635) and reduced NAC (at 3 and 10 mg/kg) DA.

Homogeneity in the 5-HT_{1A} receptor regulation of frontal cortical monoamine release between species is suggested by a study in mice (as compared to previous rat studies) which demonstrated decreases in 5-HT and increases in DA release with systemic 8-OH-DPAT or MKC-242 administration, which were 5-HT_{1A} receptor dependent [281]. However intra-PFC perfusion with 8-OH-DPAT increased DA release without affecting PFC 5-HT release.

Relevance of the 5-HT_{1A} Receptor Regulation of PFC Dopamine Release to Schizophrenia

The preferential ability to increase PFC synaptic DA (*vs.* striatal DA) has been proposed to differentiate between the atypical APDs such as clozapine, olanzapine, risperidone and ziprasidone from typical APDs such as haloperidol. Both clozapine and ziprasidone are 5-HT_{1A} (partial) agonists and their ability to increase PFC DA levels is partially reversed in the presence of WAY100,635 [301]. In addition, WAY100,635 can also partially attenuate quetiapine-, iloperidone- and melperone-induced increases in dialysate PFC DA levels which is interesting given the known D_2 / $5HT_{2A}$ antagonist profile of these APDs [301]. Other work by the same group has demonstrated that the increase in PFC DA levels associated with the selective 5-HT_{2A} antagonist MDL100,907 are also reversed by WAY100,635 [302]. Taken together, this suggests that direct (or indirect) activation of 5-HT_{1A} receptors may be important in regulating PFC DA release and by extension, antipsychotic activity and there is a 5-HT_{2A} – 5-HT_{1A} receptor interplay controlling cortical DA levels following antipsychotic administration.

5-HT_{1A} receptor agonism improves attentional processing in the mPFC [250] which is important for the treatment of schizophrenia and suggests that direct, or even indirect, 5-HT_{1A} agonist-like effects in the mPFC will improve attentional processing in schizophrenic patients. Clinical studies have demonstrated that the 5-HT_{1A} partial agonist tandospirone improved cognition in schizophrenic patients [251,252].

Thus, there is compelling evidence that 5-HT_{1A} receptor agonism, either direct or indirect, may help with at least some of the symptom sets of schizophrenia.

5-$HT_{1B/D}$ Receptor Regulation of Dopamine Transmission

Only one study has examined the contribution of the 5-$HT_{1B/D}$ receptor to DA neurotransmission in the PFC. Local (intra-PFC) application of 5-HT was associated with a concentration-dependent increase in dialysate PFC DA levels which was completely blocked in the presence of the selective 5-$HT_{1B/1D}$ antagonist GR127935 [270]. Similarly, infusions with the 5-HT_{1B} receptor agonists CP93,129 and CP94,253 augmented mPFC DA and which was antagonized by GR127935 administration [270]. Similarly, the 5-$HT_{1B/D}$ regulation of DA release at the levels of the VTA and NAC has been characterized; intra-VTA perfusion of CP93,129 concentration-dependently increased both local and NAC dialysate DA levels. Co-perfusion of CP93,129 with the 5-HT_{1B} receptor antagonist SB216641 or the 5-$HT_{1D/1A}$ receptor antagonist BRL15572 antagonized both the effects on VTA and NAC DA levels while co-perfusion with the 5-HT_{1A} antagonist WAY100,635 did not alter the CP93,129 mediated increase in both VTA and NAC DA levels [303], suggesting that VTA 5-$HT_{1B/D}$ receptors regulate mesolimbic DA release. Whether VTA 5-$HT_{1B/D}$ receptors also regulate PFC DA release has not yet been established.

5-$HT_{2A/2C}$ Receptor Regulation of Dopamine Transmission in the Prefrontal Cortex

Recent studies have demonstrated stimulatory modulatory actions on DA release following systemic administration of $5HT_{2A/2C}$ antagonists in the mPFC. The selective $5HT_{2A}$ antagonist M100,907 alone had no effect on PFC DA levels while the 5-$HT_{2A/2C}$ antagonist SR46349-B increased PFC DA release [304]. SR46349-B potentiated haloperidol-induced DA release in the PFC and NAC, but MDL100,907 potentiated haloperidol-induced mPFC DA release [280]. Furthermore, the 5-HT_{1A} receptor antagonist WAY100,635 was reported to antagonize the effects of haloperidol plus MDL100,907 as well as SR46349-B on DA release in the mPFC [304]. Taken together, this suggests that 5-HT_{2A} and 5-HT_{2C} antagonism together with D_2 receptor antagonism may potentiate mPFC DA release *via* 5-HT_{1A} receptor stimulation. Local administration of MDL100,907 into the mPFC blocked both DA release stimulated by the 5-HT_2 receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and by K^+-stimulation suggesting that PFC 5-HT_{2A} receptor stimulation potentiates the phasic release of mesocortical DA [305]. The ability of systemic MDL100,907 to potentiate the haloperidol-induced increase in PFC DA release may therefore rely on non-PFC 5-HT_{2A} receptors [280,304].

The 5-HT_2 agonist DOI potentiated amphetamine induced PFC DA release. This effect of DOI was completely prevented by MDL100,907 which by itself had no effect on basal or amphetamine-induced DA release [306], suggesting that the MDL100,907 potentiation of haloperidol-induced DA release in the PFC differs from that induced by amphetamine, where no potentiation was seen. This may reflect a dynamic interplay between D_2 and 5-HT_{2A} receptor populations but it is unclear why such differences occur between amphetamine-stimulated and D_2 antagonist-induced changes but perhaps this is related to the tone created by each manipulation. For example, amphetamine-stimulated release is an extremely strong stimulus and when sulpiride doses are high there is a lack of interaction with the 5-HT_{1A} receptor [248], suggesting that at high levels of DA stimulation the effect is lost (or perhaps masked). This appears to be supported by the fact that the greatest effects have been noted with haloperidol at lower doses that produce less than 80% of DA D_2 receptor occupation (see [307]).

Both systemic and intra-VTA but not intra-PFC administration of the 5-HT_{2C} agonist Ro60-0175 antagonized stress-induced PFC DA release without altering basal DA levels [308]. Nonetheless, administration of the 5-HT_{2C} antagonist SB242084 reversed the Ro60-0175-induced DA changes in the VTA and were associated with an increased PFC DA level suggesting that endogenous 5-HT tonically acts on VTA 5-HT_{2C} receptors to inhibit DA release in the PFC. Studies infusing the 5-$HT_{2C/2B}$ receptor antagonist SB206553 into the mPFC *via* reverse microdialysis failed to demonstrate any local effects on synaptic DA under normal or high K^+ conditions, although similar administration in the dorsal striatum augmented striatal DA levels [309].

Electrophysiological studies have suggested that the electrical stimulation of the MRN causes an inhibition of prefrontal neurones and that these effects are inhibited by 5-HT_2 receptor antagonism (RP62203). However, the inhibitory effect mediated by electrical stimulation of the VTA was not changed by RP62203 suggesting that serotonergic but not dopaminergic afferents are under the control of 5-HT_2 receptors [310] which will impact dopaminergic function in the PFC. Certainly more recent electrophysiological studies suggest that 5-HT_{2A} receptors modulate the excitatory post-synaptic potentials of layer V cortical neurones (for review see [311]), an area involved with higher order cognitive function and the aetiology of schizophrenia and abuse. Layer V is also associated with electrophysiological responses insofar as APDs such as haloperidol increase burst firing under conditions of reduced glutamatergic tone [312]. However, specific interactions between serotonin and DA and receptors have not been addressed at this level to the best of the authors' knowledge.

Contribution of $5HT_{2A/2C}$ Receptor Blockade to Antipsychotic Activity

The suggestion that 5-HT_{2A} and/or 5-HT_{2C} receptor blockade contributes to antipsychotic activity has been proposed by a number of authors (see [307]). Much of the data supporting this concept comes from atypical antipsychotic agents which possess higher affinity at the 5-HT_{2A} receptor than at the DA D_2 receptor and the ability of atypical APDs to preferentially augment DA in the mPFC (*vs.* striatum) [287-291] and it appears that 5-HT_{2A} receptor antagonism is, at least in part, responsible for this action [288]. In addition, it has been shown *in vitro* that 5-$HT_{2A/2C}$ receptors enhance D_2 receptor-mediated auto-inhibition of DA neurons in the VTA (and not the SN) [313], which may also contribute to the efficacy of atypical APDs.

Local application of DOI by microiontophoresis in the PFC inhibited the cell firing which was antagonized by APDs such as clozapine and spiperone, which, among other receptor functionalities, possess 5-HT_{2A} receptor antagonist properties. In contrast, the typical APDs haloperidol and L-sulpiride did not antagonize the effect of DOI on PFC cell firing, suggesting that 5-$HT_{2A/2C}$ blockade contributes to the

mechanism of action of clozapine and spiperone [314,315]. Studies with clozapine and amperozide demonstrated preferential enhancement of PFC DA release while risperidone (a mixed D_2 receptor antagonist and 5-HT_2 receptor antagonist) increased DA levels to the same extent in the PFC, NAC and striatum [316]. Interestingly DOI but not Ro60-0175 attenuated the increase in PFC DA levels associated with clozapine suggesting that 5-HT_{2A} receptor blockade regulates in part the clozapine-induced increase in PFC DA release [302]. SB 242084 (a 5-HT_{2C} receptor antagonist) increased DA levels in the mPFC [317] whereas 5-HT_{2A} receptor antagonists will only do this in the presence of (weak) DA D_2 receptor antagonism (see above) and this might be one of the reasons why SR46349-B augments DA when given alone [304] and why this compound possesses putative antipsychotic activity superior to the action to that of the selective 5-HT_{2A} receptor antagonist MDL100,907 [307]. Indeed, recent evidence suggests that 5-HT_{2C} receptor polymorphisms may be important for positive treatment outcome on negative but not positive symptoms [318]. However, it needs to be considered that polymorphisms of the 5-HT_{2C} receptor can result in antipsychotic-induced weight gain [319,320].

5-HT_3 Receptor Regulation of Dopamine Transmission in the Prefrontal Cortex

A number of studies have reported facilitatory effects on PFC DA release by 5-HT_3 receptor activation in the rat PFC which is impulse and Ca^{2+} dependent [321,322]. Local PFC administration of n-methylquipazine (NMQ), a putative 5-HT_3 receptor agonist in the PFC increased extracellular DA levels and decreased extracellular DOPAC levels. Interestingly, local administration of the 5-HT_3 antagonist BRL46470A had the reverse effect, a concentration-dependent decrease in extracellular PFC DA levels. However neither BRL46470A or the 5-HT_2 antagonist MDL100,907 could attenuate the increased PFC DA levels induced by NMQ [321]. Pretreatment with the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine attenuated the NMQ-induced increase in DA levels, suggesting that the elevation by NMQ of DA levels is dependent on newly synthesized stores of DA. Care must be taken in interpretating the effects of NMQ on PFC DA levels since mechanisms other than 5-HT_3 agonism may be involved, such as changes in DA turnover.

Microiontophoretic application of the 5-HT_3 receptor agonist 2-methyl-5-HT produced a current-dependent inhibition of the basal firing rate of PFC cells in sham- and 5,7-dihydroxytryptamine-lesioned rats which was of greater magnitude in lesioned animals [323,324]. Administration of the SSRI fluoxetine was associated with an increase in extracellular PFC DA levels. Both local and systemic pre-treatment with the 5-HT_3 antagonist ICS205930 blocked this effect in the PFC suggesting that fluoxetine increases extracellular PFC DA levels by stimulating local 5-HT_3 receptors indirectly [325]. So possibly the inhibitory action of DA in the PFC is dependent upon 5-HT acting on local 5-HT_3 receptors.

This inter-relationship between DA and 5-HT tone and 5-HT_3 receptor expression and functionality has not been limited to studies in which lesions of the 5-HT system have taken place. In animals with unilateral medial forebrain bundle 6-hydroxy-DA lesions, which lesions DA afferents, binding densities of the selective 5-HT_3 antagonist [^{3}H]GR65630 were reduced in PFC and entorhinal cortex on the lesioned side of the rat brain compared to the control tissues. Furthermore, an increase in affinity for [^{3}H]GR65630 binding was observed in the PFC but not in other regions such as the amygdala or hippocampus [326], suggesting that 5-HT_3 receptor expression and functionality is dependent on dopaminergic tone.

Contribution of 5-HT_3 Receptors to Antipsychotic Activity

The relevance of 5-HT_3 mediated effects on dopaminergic transmission has been studied by determining the effects of both dopaminergic and 5-HT_3 ligands on PFC cell firing. The atypical APD clozapine (and RMI81,582) could reverse the inhibition produced by DA and by the 5-HT_3 receptor agonists phenylbiguanide and 2-methyl-5-HT, although cautious conclusions should be drawn due to the "rich" pharmacology of clozapine [314], suggesting a 5-HT_3 antagonist nature of clozapine [327].

The ability of 5-HT_3 receptors to gate the inhibitory action of the mesocorticolimbic (*vs.* nigrostriatal) DA system may account for the higher efficacy of atypical APDs with 5-HT_3 antagonistic activity (e.g. clozapine) in treating schizophrenic symptoms (for review see [328]).

5-HT_4 Receptor Regulation of Dopaminergic Transmission

To date, no studies have reported the influence of the 5-HT_4 receptor on DA transmission in the PFC. However, the regulation of striatal DA release by the 5-HT_4 receptor has been demonstrated by several independent groups [329-331]. The ability of locally applied 5-HT to elevate striatal DA levels has also been shown to be dependent upon the 5-HT_4 receptor, being reversed in the presence of the 5-HT_4 receptor antagonist GR125487 or DAU6285 (a 5-$HT_{3/4}$ antagonist) [331]. Local (intra-striatal) perfusion with the 5-HT_4 receptor agonists, renzapride and (S)-zacopride also elevated dialysate striatal DA levels which were completely antagonized by the 5-HT_4 receptor antagonists SDZ205-557 and GR113808 [330]. In addition, the morphine-induced increase in striatal DA release was reversed in the presence of the 5-HT_4 receptor antagonists GR125487 and SB204070 and potentiated in the presence of the 5-HT_4 agonist prucalopride [329]. It is therefore possible that either direct or indirect 5-HT_4 receptor-mediated mechanisms might have a role in the regulation of mPFC DA neurotransmission. Interestingly, no 5-HT_4 receptor regulation is apparent with respect to morphine-induced increase in NAC DA release. This may however reflect an interaction between opiate receptors and the 5-HT_4 receptor as 5-HT_4 ligands fail to influence amphetamine- and cocaine-induced DA release in these regions. [329,332]

5-HT_6 Receptor Regulation of Dopamine Transmission in the Prefrontal Cortex

Due to the paucity of selective 5-HT_6 receptor agonists, no study has thus far characterized the effect of 5-HT_6 receptor stimulation. However, the administration of the

selective 5-HT_6 receptor antagonist SB271046 increased synaptic DA and NA levels without altering 5-HT neurotransmission in the PFC [333]. In another study the effects of the 5-HT_6 antagonist SB258510A on psychostimulant-induced locomotor activity, cocaine self-administration and increased extracellular DA levels in the frontal cortex and NAC were investigated [334]. The locomotor-activating effects of amphetamine were dose-dependently enhanced by pre-treatment with SB258510A. Similarly, amphetamine self-administration was dose-dependently altered by SB258510A in a manner indicative of enhanced reinforcing effects of amphetamine on both fixed and progressive ratio schedules of reinforcement. SB258510A treatment had no effect on either cocaine-induced locomotor activity or cocaine self-administration. Dual-probe *in vivo* microdialysis revealed that pre-treatment with SB258510A potentiated an amphetamine-induced increase in extracellular DA, more robustly in the mPFC than in the NAC indicating that activation of 5-HT_6 receptors may regulate behaviours related to amphetamine, and point to the mPFC as a possible site of action for these effects. Such effects may link with positive symptoms, but further studies will be needed to elucidate the role of 5-HT_6 receptors fully on mPFC DA neurotransmission.

Dopamine-Serotonin Interactions in Schizophrenia

There is considerable evidence for the involvement of brain dopaminergic and serotonergic systems in (medicated) schizophrenia neuropathology [335]. Alterations in the expression of DA D_2 receptors in the caudate-putamen, prefrontal, perirhinal and temporal cortices were observed in medicated post-mortem schizophrenic brain compared to non-schizophrenic controls. These changes include altered laminar distribution of the DA D_2 receptor and modified modular organization of D_2 receptors in the superior temporal gyrus. However, post-mortem studies suffer from the difficulty to separate the effects of chronic exposure to APDs from that of the disease process. In medication-free schizophrenics, elevations of D_3 receptors in target regions of the mesolimbic DA system were highly correlated with elevated 5-HT_{1A} receptor expression in PFC. In contrast, APD treatment was correlated with a reduction of D_3 receptors in the NAC.

Abnormalities in DA neurotransmission in the PFC have been implicated in the pathophysiology of schizophrenia. The integrity of the DA projections to the PFC in schizophrenic subjects is reduced by one-third in the deep layers (V/VI) of the dorsolateral PFC (Brodmann area 9) demonstrated by the reduction of tyrosine hydroxylase (TH) and DA membrane transporter immunoreactivity [336]. Furthermore, primate studies have shown that the density of TH immunoreactivity was unchanged following chronic treatment with the D_2 receptor antagonist and typical APD haloperidol suggesting that APDs may not exert their effects *via* alteration of TH expression and that TH changes seen in patients may be a consequence of disease and not treatment.

The density of 5-HT uptake sites was unchanged in schizophrenia [336], 5-HT tone in these regions could be unaltered. However, in medicated schizophrenics, the density of 5-$HT_{2A/2C}$ receptors was decreased in Brodmann areas 24 and 6 irrespective of their antipsychotic treatment history [337]. Interestingly, the ability of APDs to decrease 5-$HT_{2A/2C}$ receptor binding may be linked to their therapeutic effect as unmedicated schizophrenics did not demonstrate this reduction in 5-$HT_{2A/2C}$ expression. While the density of 5-HT_{1A} receptor expression was elevated in Brodmann areas 24, 9a, 44 and 6 in schizophrenics, this did not appear to be related to APD treatment. Taken together, this suggests a dynamic interplay between the expression of DA and 5-HT receptors in schizophrenia and by APD treatment, making it difficult to draw firm conclusions. Moreover, a PET study in unmedicated patients failed to demonstrate changes in 5-HT_{2A} receptor density [338].

In summary, serotonergic – dopaminergic interactions play a key role in the regulations of mPFC dopaminergic function. More particularly 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} receptors appear pivotal in interactions of APDs with mPFC dopaminergic levels. Increasing dopaminergic levels in the mPFC in schizophrenic patients may be pivotal in treating the symptoms of schizophrenia [246,249] and the actions of 5-HT_{1A} agonists and 5-HT_2 receptor antagonists might be of key importance in improving symptomatologies in this patient-set. However, further studies will be needed to fully elucidate the role of other serotonergic receptor subtypes.

ABBREVIATIONS

APD	=	Antipsychotic drug
cAMP	=	Cyclic adenosine monophosphate
DA	=	Dopamine
DARPP-32	=	Dopamine and cyclic AMP-regulated phosphoprotein, 32 kDa
DOI	=	1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane
DOPAC	=	3,4-Dihydroxy-phenylacetic acid
DRN	=	Dorsal raphe nucleus
GDP	=	Guanosine diphosphate
GIRK	=	G protein-coupled inward rectifying K^+
GPCR	=	G protein-coupled receptor
GRK	=	G protein-coupled receptor kinases
GTP	=	Guanosine triphosphate
5-HT	=	5-Hydroxy-tryptamine
HVA	=	Homovanillic acid
L-DOPA	=	3,4-Dihydroxy-L-phenylalanine
MAP	=	Mitogen-activated protein
MDMA	=	3,4-Methylenedioxy-methamphetamine
MRN	=	Median raphe nucleus
NAC	=	Nucleus accumbens
NMQ	=	N-methylquipazine
8-OH-DPAT	=	8-Hydroxy-2(di-n-propylamnio)tetralin
PET	=	Positron emission tomography
PFC	=	Prefrontal cortex
PKA	=	Protein kinase A

PKC = Protein kinase C

PLA_2 = Phospholipase A_2

PLC = Phospolipase C

PLD = Phospolipase D

RGS = Regulator of G protein signalling

RLS = Restless legs syndrome

SN = Substantia nigra

SSRI = serotonin Specific reuptake inhibitor

TH = Tyrosine hydroxylase

7TM = Seven hydrophobic trans-membrane spanning domains

VTA = Ventral tegmental area

REFERENCES

[1] Missale, C.; Nash, S.R.; Robinson, S.W.; Jaber, M.; Caron, M.G. *Physiol. Rev.*, **1998**, *78*, 189-225.

[2] Vallone, D.; Picetti, R.; Borrelli, E. *Neurosci. Biobehav. Rev.*, **2000**, *24*, 125-32.

[3] Kebabian, J.W.; Calne, D.B. *Nature*, **1979**, *277*, 93-6.

[4] Bunzow, J.R.; Van Tol, H.H.; Grandy, D.K.; Albert, P.; Salon, J.; Christie, M.; Machida, C.A.; Neve, K.A.; Civelli, O. *Nature*, **1988**, *336*, 783-7.

[5] Dal Toso, R.; Sommer, B.; Ewert, M.; Herb, A.; Pritchett, D.B.; Bach, A.; Shivers, B.D.; Seeburg, P.H. *EMBO J.*, **1989**, *8*, 4025-34.

[6] Giros, B.; Sokoloff, P.; Martres, M.P.; Riou, J.F.; Emorine, L.J.; Schwartz, J.C. *Nature*, **1989**, *342*, 923-6.

[7] Monsma, F.J. Jr.; McVittie, L.D.; Gerfen, C.R.; Mahan, L.C.; Sibley, D.R. *Nature*, **1989**, *342*, 926-9.

[8] Gejman, P.V.; Ram, A.; Gelernter, J.; Friedman, E.; Cao, Q.; Pickar, D.; Blum, K.; Noble, E.P.; Kranzler, H.R.; O'Malley, S.; *et al. JAMA*, **1994**, *271*, 204-8.

[9] Centonze, D.; Usiello, A.; Gubellini, P.; Pisani, A.; Borrelli, E.; Bernardi, G.; Calabresi, P. *Neuropsychopharmacology*, **2002**, *27*, 723-6.

[10] Wang, Y.; Xu, R.; Sasaoka, T.; Tonegawa, S.; Kung, M.P.; Sankoorikal, E.B. *J. Neurosci.*, **2000**, *20*, 8305-14.

[11] Usiello, A.; Baik, J.H.; Rouge-Pont, F.; Picetti, R.; Dierich, A.; LeMeur, M.; Piazza, P.V.; Borrelli, E. *Nature*, **2000**, *408*, 199-203.

[12] Lindgren, N.; Usiello, A.; Goiny, M.; Haycock, J.; Erbs, E.; Greengard, P.; Hokfelt, T.; Borrelli, E.; Fisone, G. *Proc. Natl. Acad. Sci. U. S. A.*, **2003**, *100*, 4305-9.

[13] Sokoloff, P.; Giros, B.; Martres, M.P.; Bouthenet, M.L.; Schwartz, J.C. *Nature*, **1990**, *347*, 146-51.

[14] Van Tol, H.H.; Bunzow, J.R.; Guan, H.C.; Sunahara, R.K.; Seeman, P.; Niznik, H.B.; Civelli, O. *Nature*, **1991**, *350*, 610-4.

[15] Jackson, D.M.; Westlind-Danielsson, A. *Pharmacol. Ther.*, **1994**, *64*, 291-370.

[16] Snyder, L.A.; Roberts, J.L.; Sealfon, S.C. *Biochem. Biophys. Res. Commun.*, **1991**, *180*, 1031-5.

[17] Fishburn, C.S.; Belleli, D.; David, C.; Carmon, S.; Fuchs, S. *J. Biol. Chem.*, **1993**, *268*, 5872-8.

[18] Elmhurst, J.L.; Xie, Z.; O'Dowd, B.F.; George, S.R. *Brain Res. Mol. Brain Res.*, **2000**, *80*, 63-74.

[19] Van Tol, H.H.; Wu, C.M.; Guan, H.C.; Ohara, K.; Bunzow, J.R.; Civelli, O.; Kennedy, J.; Seeman, P.; Niznik, H.B.; Jovanovic, V. *Nature*, **1992**, *358*, 149-52.

[20] Seeman, P.; Van Tol, H.H. *Trends Pharmacol. Sci.*, **1994**, *15*, 264-70.

[21] Sunahara, R.K.; Niznik, H.B.; Weiner, D.M.; Stormann, T.M.; Brann, M.R.; Kennedy, J.L.; Gelernter, J.E.; Rozmahel, R.; Yang, Y.L.; Israel, Y.; Seeman, P.; O'Dowd, B.F. *Nature*, **1990**, *347*, 80-3.

[22] Monsma, F.J. Jr.; Mahan, L.C.; McVittie, L.D.; Gerfen, C.R.; Sibley, D.R. *Proc. Natl. Acad. Sci. U. S. A.*, **1990**, *87*, 6723-7.

[23] Dearry, A.; Gingrich, J.A.; Falardeau, P.; Fremeau, R.T. Jr.; Bates, M.D.; Caron, M.G. *Nature*, **1990**, *347*, 72-6.

[24] Zhou, Q.Y.; Grandy, D.K.; Thambi, L.; Kushner, J.A.; Van Tol, H.H.; Cone, R.; Pribnow, D.; Salon, J.; Bunzow, J.R.; Civelli, O. *Nature*, **1990**, *347*, 76-80.

[25] Sunahara, R.K.; Guan, H.C.; O'Dowd, B.F.; Seeman, P.; Laurier, L.G.; Ng, G.; George, S.R.; Torchia, J.; Van Tol, H.H.; Niznik, H.B. *Nature*, **1991**, *350*, 614-9.

[26] Tiberi, M.; Jarvie, K.R.; Silvia, C.; Falardeau, P.; Gingrich, J.A.; Godinot, N.; Bertrand, L.; Yang-Feng, T.L.; Fremeau, R.T. Jr.; Caron, M.G. *Proc. Natl. Acad. Sci. U. S. A.*, **1991**, *88*, 7491-5.

[27] Weinshank, R.L.; Adham, N.; Macchi, M.; Olsen, M.A.; Branchek, T.A.; Hartig, P.R. *J. Biol. Chem.*, **1991**, *266*, 22427-35.

[28] Sidhu, A.; Niznik, H.B. *Int. J. Dev. Neurosci.*, **2000**, *18*, 669-77.

[29] Gether, U. *Endocr. Rev.*, **2000**, *21*, 90-113.

[30] Gingrich, J.A.; Caron, M.G. *Annu. Rev. Neurosci.*, **1993**, *16*, 299-321.

[31] Civelli, O.; Bunzow, J.R.; Grandy, D.K. *Annu. Rev. Pharmacol. Toxicol.*, **1993**, *33*, 281-307.

[32] Shi, L.; Javitch, J.A. *Annu. Rev. Pharmacol. Toxicol.*, **2002**, *42*, 437-67.

[33] Kalani, M.Y.; Vaidehi, N.; Hall, S.E.; Trabanino, R.J.; Freddolino, P.L.; Kalani, M.A.; Floriano, W.B.; Kam, V.W.; Goddard, W.A., 3rd *Proc. Natl. Acad. Sci. U. S. A.*, **2004**, *101*, 3815-20.

[34] Shi, L.; Javitch, J.A. *Proc. Natl. Acad. Sci. U. S. A.*, **2004**, *101*, 440-5.

[35] Dohlman, H.G.; Caron, M.G.; DeBlasi, A.; Frielle, T.; Lefkowitz, R.J. *Biochemistry*, **1990**, *29*, 2335-42.

[36] Jin, H.; Xie, Z.; George, S.R.; O'Dowd, B.F. *Eur. J. Pharmacol.*, **1999**, *386*, 305-12.

[37] Ferguson, S.S. *Pharmacol. Rev.*, **2001**, *53*, 1-24.

[38] Kohout, T.A.; Lefkowitz, R.J. *Mol. Pharmacol.*, **2003**, *63*, 9-18.

[39] Sibley, D.R.; Neve, K.A., In *The Dopamine Receptors*, Neve, K.A. and Neve, R.L., Ed.; Humana Press: Totowa, New Jersey, **1997**; pp. 383-424.

[40] Kenakin, T. *Annu. Rev. Pharmacol. Toxicol.*, **2002**, *42*, 349-79.

[41] Gurevich, V.V.; Gurevich, E.V. *Trends Pharmacol. Sci.*, **2004**, *25*, 105-11.

[42] Gardner, B.; Liu, Z.F.; Jiang, D.; Sibley, D.R. *Mol. Pharmacol.*, **2001**, *59*, 310-21.

[43] Jiang, D.; Sibley, D.R. *Mol. Pharmacol.*, **1999**, *56*, 675-83.

[44] Jackson, A.; Iwasiow, R.M.; Chaar, Z.Y.; Nantel, M.F.; Tiberi, M. *J. Neurochem.*, **2002**, *82*, 683-97.

[45] Tiberi, M.; Nash, S.R.; Bertrand, L.; Lefkowitz, R.J.; Caron, M.G. *J. Biol. Chem.*, **1996**, *271*, 3771-8.

[46] Mason, J.N.; Kozell, L.B.; Neve, K.A. *Mol. Pharmacol.*, **2002**, *61*, 806-16.

[47] Lamey, M.; Thompson, M.; Varghese, G.; Chi, H.; Sawzdargo, M.; George, S.R.; O'Dowd, B.F. *J. Biol. Chem.*, **2002**, *277*, 9415-21.

[48] Kim, O.J.; Gardner, B.R.; Williams, D.B.; Marinec, P.S.; Cabrera, D.M.; Peters, J.D.; Mak, C.C.; Kim, K.M.; Sibley, D.R. *J. Biol. Chem.*, **2004**, *279*, 7999-8010.

[49] Ito, K.; Haga, T.; Lameh, J.; Sadee, W. *Eur. J. Biochem.*, **1999**, *260*, 112-9.

[50] Kim, K.M.; Valenzano, K.J.; Robinson, S.R.; Yao, W.D.; Barak, L.S.; Caron, M.G. *J. Biol. Chem.*, **2001**, *276*, 37409-14.

[51] Namkung, Y.; Sibley, D.R. *J. Biol. Chem.*, **2004**, *279*, 49533-41.

[52] Kabbani, N.; Jeromin, A.; Levenson, R. *Cell. Sign.*, **2004**, *16*, 497-503.

[53] Jeanneteau, F.; Diaz, J.; Sokoloff, P.; Griffon, N. *Mol. Biol. Cell*, **2004**, *15*, 696-705.

[54] Angers, S.; Salahpour, A.; Bouvier, M. *Annu. Rev. Pharmacol. Toxicol.*, **2002**, *42*, 409-35.

[55] Devi, L.A. *Trends Pharmacol. Sci.*, **2001**, *22*, 532-7.

[56] Bai, M. *Cell. Sign.*, **2004**, *16*, 175-86.

[57] Hansen, J.L.; Sheikh, S.P. *Eur. J. Pharm. Sci.*, **2004**, *23*, 301-17.

[58] George, S.R.; O'Dowd, B.F.; Lee, S.P. *Nat. Rev. Drug.Discov.*, **2002**, *1*, 808-20.

[59] Terrillon, S.; Bouvier, M. *EMBO Rep.*, **2004**, *5*, 30-4.

[60] Armstrong, D.; Strange, P.G. *J. Biol. Chem.*, **2001**, *276*, 22621-9.

[61] Zawarynski, P.; Tallerico, T.; Seeman, P.; Lee, S.P.; O'Dowd, B.F.; George, S.R. *FEBS Lett.*, **1998**, *441*, 383-6.

[62] Ng, G.Y.; O'Dowd, B.F.; Lee, S.P.; Chung, H.T.; Brann, M.R.; Seeman, P.; George, S.R. *Biochem. Biophys. Res. Commun.*, **1996**, *227*, 200-4.

[63] Lee, S.P.; O'Dowd, B.F.; Ng, G.Y.; Varghese, G.; Akil, H.; Mansour, A.; Nguyen, T.; George, S.R. *Mol. Pharmacol.*, **2000**, *58*, 120-8.

[64] Logan, J.; Fowler, J.S.; Dewey, S.L.; Volkow, N.D.; Gatley, S.J. *J. Neural Transm.*, **2001**, *108*, 279-86.
[65] Guo, W.; Shi, L.; Javitch, J.A. *J. Biol. Chem.*, **2003**, *278*, 4385-8.
[66] Lee, S.P.; O'Dowd, B.F.; Rajaram, R.D.; Nguyen, T.; George, S.R. *Biochemistry*, **2003**, *42*, 11023-31.
[67] Karpa, K.D.; Lin, R.; Kabbani, N.; Levenson, R. *Mol. Pharmacol.*, **2000**, *58*, 677-83.
[68] Nimchinsky, E.A.; Hof, P.R.; Janssen, W.G.; Morrison, J.H.; Schmauss, C. *J. Biol. Chem.*, **1997**, *272*, 29229-37.
[69] George, S.R.; Lee, S.P.; Varghese, G.; Zeman, P.R.; Seeman, P.; Ng, G.Y.; O'Dowd, B.F. *J. Biol. Chem.*, **1998**, *273*, 30244-8.
[70] Ng, G.Y.; Trogadis, J.; Stevens, J.; Bouvier, M.; O'Dowd, B.F.; George, S.R. *Proc. Natl. Acad. Sci. U. S. A.*, **1995**, *92*, 10157-61.
[71] Maggio, R.; Scarselli, M.; Novi, F.; Millan, M.J.; Corsini, G.U. *J. Neurochem.*, **2003**, *87*, 631-41.
[72] Scarselli, M.; Novi, F.; Schallmach, E.; Lin, R.; Baragli, A.; Colzi, A.; Griffon, N.; Corsini, G.U.; Sokoloff, P.; Levenson, R.; Vogel, Z.; Maggio, R. *J. Biol. Chem.*, **2001**, *276*, 30308-14.
[73] Canals, M.; Marcellino, D.; Fanelli, F.; Ciruela, F.; de Benedetti, P.; Goldberg, S.R.; Neve, K.; Fuxe, K.; Agnati, L.F.; Woods, A.S.; Ferre, S.; Lluis, C.; Bouvier, M.; Franco, R. *J. Biol. Chem.*, **2003**, *278*, 46741-9.
[74] Gines, S.; Hillion, J.; Torvinen, M.; Le Crom, S.; Casado, V.; Canela, E.I.; Rondin, S.; Lew, J.Y.; Watson, S.; Zoli, M.; Agnati, L.F.; Verniera, P.; Lluis, C.; Ferre, S.; Fuxe, K.; Franco, R. *Proc. Natl. Acad. Sci. U. S. A.*, **2000**, *97*, 8606-11.
[75] Rocheville, M.; Lange, D.C.; Kumar, U.; Patel, S.C.; Patel, R.C.; Patel, Y.C. *Science*, **2000**, *288*, 154-7.
[76] Rimondini, R.; Fuxe, K.; Ferre, S. *Neuroreport*, **1999**, *10*, 2051-4.
[77] Werkman, T.R.; Kruse, C.G.; Nievelstein, H.; Long, S.K.; Wadman, W.J. *Neuroscience*, **2000**, *95*, 417-23.
[78] Franco, R.; Ferre, S.; Agnati, L.; Torvinen, M.; Gines, S.; Hillion, J.; Casado, V.; Lledo, P.; Zoli, M.; Lluis, C.; Fuxe, K. *Neuropsychopharmacology*, **2000**, *23*, S50-9.
[79] Fuxe, K.; Ferre, S.; Zoli, M.; Agnati, L.F. *Brain Res. Brain Res. Rev.*, **1998**, *26*, 258-73.
[80] Ferre, S.; Ciruela, F.; Canals, M.; Marcellino, D.; Burgueno, J.; Casado, V.; Hillion, J.; Torvinen, M.; Fanelli, F.; Benedetti Pd, P.; Goldberg, S.R.; Bouvier, M.; Fuxe, K.; Agnati, L.F.; Lluis, C.; Franco, R.; Woods, A. *Parkinsonism Relat. Disord.*, **2004**, *10*, 265-71.
[81] Tate, C.G.; Grisshammer, R. *Trends Biotechnol.*, **1996**, *14*, 426-30.
[82] Bockaert, J.; Fagni, L.; Dumuis, A.; Marin, P. *Pharmacol. Ther.*, **2004**, *103*, 203-21.
[83] Neves, S.R.; Ram, P.T.; Iyengar, R. *Science*, **2002**, *296*, 1636-9.
[84] Patel, T.B. *Pharmacol. Rev.*, **2004**, *56*, 371-85.
[85] Hamm, H.E. *J. Biol. Chem.*, **1998**, *273*, 669-72.
[86] Cabrera-Vera, T.M.; Vanhauwe, J.; Thomas, T.O.; Medkova, M.; Preininger, A.; Mazzoni, M.R.; Hamm, H.E. *Endocr. Rev.*, **2003**, *24*, 765-81.
[87] Chidiac, P.; Roy, A.A. *Receptors Channels*, **2003**, *9*, 135-47.
[88] Neve, K.A.; Seamans, J.K.; Trantham-Davidson, H. *J. Recept. Signal Transduct. Res.*, **2004**, *24*, 165-205.
[89] Kenakin, T. *Trends Pharmacol. Sci.*, **1995**, *16*, 232-8.
[90] Hermans, E. *Pharmacol. Ther.*, **2003**, *99*, 25-44.
[91] Gazi, L.; Nickolls, S.A.; Strange, P.G. *Br. J. Pharmacol.*, **2003**, *138*, 775-86.
[92] Nickolls, S.A.; Strange, P.G. *Neuropharmacology*, **2004**, *47*, 860-72.
[93] Gay, E.A.; Urban, J.D.; Nichols, D.E.; Oxford, G.S.; Mailman, R.B. *Mol. Pharmacol.*, **2004**, *66*, 97-105.
[94] Mirnics, K.; Middleton, F.A.; Stanwood, G.D.; Lewis, D.A.; Levitt, P. *Mol. Psychiatry*, **2001**, *6*, 293-301.
[95] Geurts, M.; Maloteaux, J.M.; Hermans, E. *Biochem. Pharmacol.*, **2003**, *66*, 1163-70.
[96] Burns, M.E.; Wensel, T.G. *Neuron*, **2003**, *38*, 853-6.
[97] Erdely, H.A.; Lahti, R.A.; Lopez, M.B.; Myers, C.S.; Roberts, R.C.; Tamminga, C.A.; Vogel, M.W. *Eur. J. Neurosci.*, **2004**, *19*, 3125-8.
[98] Rahman, Z.; Schwarz, J.; Gold, S.J.; Zachariou, V.; Wein, M.N.; Choi, K.H.; Kovoor, A.; Chen, C.K.; DiLeone, R.J.; Schwarz, S.C.; Selley, D.E.; Sim-Selley, L.J.; Barrot, M.; Luedtke, R.R.; Self, D.; Neve, R.L.; Lester, H.A.; Simon, M.I.; Nestler, E.J. *Neuron*, **2003**, *38*, 941-52.
[99] Kovoor, A.; Seyffarth, P.; Ebert, J.; Barghshoon, S.; Chen, C.K.; Schwarz, S.; Axelrod, J.D.; Cheyette, B.N.; Simon, M.I.; Lester, H.A.; Schwarz, J. *J. Neurosci.*, **2005**, *25*, 2157-65.
[100] Sunahara, R.K.; Taussig, R. *Mol. Intervent.*, **2002**, *2*, 168-84.
[101] Tasken, K.; Aandahl, E.M. *Physiol. Rev.*, **2004**, *84*, 137-67.
[102] Greengard, P.; Allen, P.B.; Nairn, A.C. *Neuron*, **1999**, *23*, 435-47.
[103] Cantrell, A.R.; Smith, R.D.; Goldin, A.L.; Scheuer, T.; Catterall, W.A. *J. Neurosci.*, **1997**, *17*, 7330-8.
[104] Berke, J.D.; Hyman, S.E. *Neuron*, **2000**, *25*, 515-32.
[105] Liu, F.C.; Graybiel, A.M. *Neuron*, **1996**, *17*, 1133-44.
[106] Konradi, C.; Cole, R.L.; Heckers, S.; Hyman, S.E. *J. Neurosci.*, **1994**, *14*, 5623-34.
[107] Watts, V.J.; Neve, K.A. *Mol. Pharmacol.*, **1997**, *52*, 181-6.
[108] Hopf, F.W.; Cascini, M.G.; Gordon, A.S.; Diamond, I.; Bonci, A. *J. Neurosci.*, **2003**, *23*, 5079-87.
[109] Phillis, J.W.; O'Regan, M.H. *Brain Res. Brain Res. Rev.*, **2004**, *44*, 13-47.
[110] Fukami, K. *J. Biochem. (Tokyo)*, **2002**, *131*, 293-9.
[111] Lezcano, N.; Mrzljak, L.; Eubanks, S.; Levenson, R.; Goldman-Rakic, P.; Bergson, C. *Science*, **2000**, *287*, 1660-4.
[112] Mahan, L.C.; Burch, R.M.; Monsma, F.J. Jr.; Sibley, D.R. *Proc. Natl. Acad. Sci. U. S. A.*, **1990**, *87*, 2196-200.
[113] Felder, C.C.; Jose, P.A.; Axelrod, J. *J. Pharmacol. Exp. Ther.*, **1989**, *248*, 171-5.
[114] Jin, L.Q.; Goswami, S.; Cai, G.; Zhen, X.; Friedman, E. *J. Neurochem.*, **2003**, *85*, 378-86.
[115] Undie, A.S.; Weinstock, J.; Sarau, H.M.; Friedman, E. *J. Neurochem.*, **1994**, *62*, 2045-8.
[116] Sidhu, A. *Mol. Neurobiol.*, **1998**, *16*, 125-34.
[117] Sidhu, A.; Kimura, K.; Uh, M.; White, B.H.; Patel, S. *J. Neurochem.*, **1998**, *70*, 2459-67.
[118] Litosch, I. *Biochem. J.*, **1996**, *319* (Pt 1), 173-8.
[119] White, B.H.; Kimura, K.; Sidhu, A. *Neuroendocrinology*, **1999**, *69*, 209-16.
[120] Kanterman, R.Y.; Mahan, L.C.; Briley, E.M.; Monsma, F.J. Jr.; Sibley, D.R.; Axelrod, J.; Felder, C.C. *Mol. Pharmacol.*, **1991**, *39*, 364-9.
[121] Lee, S.P.; So, C.H.; Rashid, A.J.; Varghese, G.; Cheng, R.; Lanca, A.J.; O'Dowd, B.F.; George, S.R. *J. Biol. Chem.*, **2004**, *279*, 35671-8.
[122] Hopf, F.W.; Mailliard, W.S.; Gonzalez, G.F.; Diamond, I.; Bonci, A. *J. Neurosci.*, **2005**, *25*, 985-9.
[123] Exton, J.H. *FEBS Lett.*, **2002**, *531*, 58-61.
[124] Everett, P.B.; Senogles, S.E. *Neurosci. Lett.*, **2004**, *371*, 34-9.
[125] Senogles, S.E. *Mol. Pharmacol.*, **2000**, *58*, 455-62.
[126] Senogles, S.E. *Mol. Cell. Endocrinol.*, **2003**, *209*, 61-9.
[127] Yang, Z.; Asico, L.D.; Yu, P.; Wang, Z.; Jones, J.E.; Bai, R.K.; Sibley, D.R.; Felder, R.A.; Jose, P.A. *Am. J. Physiol. Heart Circ. Physiol.*, **2005**, *288*, H55-61.
[128] Balboa, M.A.; Varela-Nieto, I.; Killermann Lucas, K.; Dennis, E.A. *FEBS Lett.*, **2002**, *531*, 12-7.
[129] Piomelli, D.; Pilon, C.; Giros, B.; Sokoloff, P.; Martres, M.P.; Schwartz, J.C. *Nature*, **1991**, *353*, 164-7.
[130] Chio, C.L.; Drong, R.F.; Riley, D.T.; Gill, G.S.; Slightom, J.L.; Huff, R.M. *J. Biol. Chem.*, **1994**, *269*, 11813-9.
[131] Nilsson, C.L.; Hellstrand, M.; Ekman, A.; Eriksson, E. *Br. J. Pharmacol.*, **1998**, *124*, 1651-8.
[132] Vial, D.; Piomelli, D. *J. Neurochem.*, **1995**, *64*, 2765-72.
[133] Nilsson, C.L.; Hellstrand, M.; Ekman, A.; Eriksson, E. *Life Sci.*, **1999**, *64*, 939-51.
[134] Jelsema, C.L.; Axelrod, J. *Proc. Natl. Acad. Sci. U. S. A.*, **1987**, *84*, 3623-7.
[135] Di Marzo, V.; Vial, D.; Sokoloff, P.; Schwartz, J.C.; Piomelli, D. *J. Neurosci.*, **1993**, *13*, 4846-53.
[136] Chen, N.; Appell, M.; Berfield, J.L.; Reith, M.E. *Eur. J. Pharmacol.*, **2003**, *478*, 89-95.
[137] Sweatt, J.D. *J. Neurochem.*, **2001**, *76*, 1-10.
[138] Orlowski, J.; Grinstein, S. *Pflugers Arch.*, **2004**, *447*, 549-65.
[139] Lavine, N.; Ethier, N.; Oak, J.N.; Pei, L.; Liu, F.; Trieu, P.; Rebois, R.V.; Bouvier, M.; Hebert, T.E.; Van Tol, H.H. *J. Biol. Chem.*, **2002**, *277*, 46010-9.
[140] Lacey, M.G.; Mercuri, N.B.; North, R.A. *J. Physiol. (Lond).* **1987**, *392*, 397-416.
[141] Greif, G.J.; Lin, Y.J.; Liu, J.C.; Freedman, J.E. *J. Neurosci.*, **1995**, *15*, 4533-44.

[142] Inanobe, A.; Yoshimoto, Y.; Horio, Y.; Morishige, K.I.; Hibino, H.; Matsumoto, S.; Tokunaga, Y.; Maeda, T.; Hata, Y.; Takai, Y.; Kurachi, Y. *J. Neurosci.*, **1999**, *19*, 1006-17.
[143] Davila, V.; Yan, Z.; Craciun, L.C.; Logothetis, D.; Sulzer, D. *J. Neurosci.*, **2003**, *23*, 5693-7.
[144] Surmeier, D.J.; Kitai, S.T. *Prog. Brain Res.*, **1993**, *99*, 309-24.
[145] Foehring, R.C.; Surmeier, D.J. *J. Neurophysiol.*, **1993**, *70*, 51-63.
[146] Numann, R.E.; Wadman, W.J.; Wong, R.K.S. *J. Physiol. (Lond).* **1987**, *393*, 331-53.
[147] Storm, J.F. *Prog. Brain Res.*, **1990**, *83*, 161-87.
[148] Sunahara, R.K.; Dessauer, C.W.; Gilman, A.G. *Annu. Rev. Pharmacol. Toxicol.*, **1996**, *36*, 461-80.
[149] Gorelova, N.; Seamans, J.K.; Yang, C.R. *J. Neurophysiol.*, **2002**, *88*, 3150-66.
[150] Yang, C.R.; Seamans, J.K. *J. Neurosci.*, **1996**, *16*, 1922-35.
[151] Dong, Y.; Cooper, D.; Nasif, F.; Hu, X.T.; White, F.J. *J. Neurosci.*, **2004**, *24*, 3077-85.
[152] Dong, Y.; White, F.J. *J. Neurosci.*, **2003**, *23*, 2686-95.
[153] Berridge, M.J.; Bootman, M.D.; Roderick, H.L. *Nat. Rev. Mol. Cell Biol.*, **2003**, 4, 517-29.
[154] Hille, B. *Ionic channels of excitable membranes*, Sinauer Associates: New York, **2001**
[155] Yan, Z.; Song, W.J.; Surmeier, J. *J. Neurophysiol.*, **1997**, *77*, 1003-15.
[156] Zamponi, G.W.; Snutch, T.P. *Proc. Natl. Acad. Sci. U. S. A.*, **1998**, 95, 4035-9.
[157] Hernandez-Lopez, S.; Tkatch, T.; Perez-Garci, E.; Galarraga, E.; Bargas, J.; Hamm, H.; Surmeier, D.J. *J. Neurosci.*, **2000**, *20*, 8987-95.
[158] Koga, E.; Momiyama, T. *J. Physiol. (Lond).* **2000**, *523* Pt 1, 163-73.
[159] Dunlap, K.; Luebke, J.I.; Turner, T.J. *Trends Neurosci.*, **1995**, *18*, 89-98.
[160] Cepeda, C.; Levine, M.S. *Dev. Neurosci.*, **1998**, *20*, 1-18.
[161] Lledo, P.M.; Homburger, V.; Bockaert, J.; Vincent, J.D. *Neuron*, **1992**, *8*, 455-63.
[162] Kuzhikandathil, E.V.; Oxford, G.S. *J. Neurosci.*, **1999**, *19*, 1698-707.
[163] Paupardin-Tritsch, D.; Colombaioni, L.; Deterre, P.; Gerschenfeld, H.M. *J. Neurosci.*, **1985**, *5*, 2522-32.
[164] Marchetti, C.; Carbone, E.; Lux, H.D. *Pflugers Arch.*, **1986**, *406*, 104-11.
[165] Surmeier, D.J.; Bargas, J.; Hemmings, H.C. Jr.; Nairn, A.C.; Greengard, P. *Neuron*, **1995**, *14*, 385-97.
[166] Gross, R.A.; Uhler, M.D.; Macdonald, R.L. *J. Physiol. (Lond).* **1990**, *429*, 483-96.
[167] Lai, Y.; Peterson, B.Z.; Catterall, W.A. *J. Neurochem.*, **1993**, *61*, 1333-9.
[168] Schiller, J.; Schiller, Y.; Stuart, G.; Sakmann, B. *J. Physiol. (Lond).* **1997**, *505* (Pt 3), 605-16.
[169] Seamans, J.K.; Gorelova, N.A.; Yang, C.R. *J. Neurosci.*, **1997**, *17*, 5936-48.
[170] Crill, W.E. *Annu. Rev. Physiol.*, **1996**, *58*, 349-62.
[171] Fienberg, A.A.; Hiroi, N.; Mermelstein, P.G.; Song, W.; Snyder, G.L.; Nishi, A.; Cheramy, A.; O'Callaghan, J.P.; Miller, D.B.; Cole, D.G.; Corbett, R.; Haile, C.N.; Cooper, D.C.; Onn, S.P.; Grace, A.A.; Ouimet, C.C.; White, F.J.; Hyman, S.E.; Surmeier, D.J.; Girault, J.; Nestler, E.J.; Greengard, P. *Science*, **1998**, *281*, 838-42.
[172] Calabresi, P.; Mercuri, N.; Stanzione, P.; Stefani, A.; Bernardi, G. *Neuroscience*, **1987**, *20*, 757-71.
[173] Schiffmann, S.N.; Lledo, P.M.; Vincent, J.D. *J. Physiol. (Lond).* **1995**, *483* (Pt 1), 95-107.
[174] Surmeier, D.J.; Eberwine, J.; Wilson, C.J.; Cao, Y.; Stefani, A.; Kitai, S.T. *Proc. Natl. Acad. Sci. U. S. A.*, **1992**, *89*, 10178-82.
[175] Connors, B.W.; Gutnick, M.J.; Prince, D.A. *J. Neurophysiol.*, **1982**, *48*, 1302-20.
[176] Maurice, N.; Tkatch, T.; Meisler, M.; Sprunger, L.K.; Surmeier, D.J. *J. Neurosci.*, **2001**, *21*, 2268-77.
[177] Gorelova, N.A.; Yang, C.R. *J. Neurophysiol.*, **2000**, *84*, 75-87.
[178] Gulledge, A.T.; Jaffe, D.B. *J. Neurophysiol.*, **2001**, *86*, 586-95.
[179] Ma, J.Y.; Li, M.; Catterall, W.A.; Scheuer, T. *Proc. Natl. Acad. Sci. U. S. A.*, **1994**, *91*, 12351-5.
[180] Sherrington, R.; Mankoo, B.; Attwood, J.; Kalsi, G.; Curtis, D.; Buetow, K.; Povey, S.; Gurling, H. *Genomics*, **1993**, *18*, 423-5.
[181] Grandy, D.K.; Litt, M.; Allen, L.; Bunzow, J.R.; Marchionni, M.; Makam, H.; Reed, L.; Magenis, R.E.; Civelli, O. *Am. J. Hum. Genet.*, **1989**, *45*, 778-85.
[182] Hauge, X.Y.; Grandy, D.K.; Eubanks, J.H.; Evans, G.A.; Civelli, O.; Litt, M. *Genomics*, **1991**, *10*, 527-30.
[183] Lannfelt, L.; Sokoloff, P.; Martres, M.P.; Pilon, C.; Giros, B.; Jonsson, E.; Sedvall, G.C.; J.C., S. *Psychiatr. Genet.*, **1992**, *2*, 249-56.
[184] Gelernter, J.; Pakstis, A.J.; Pauls, D.L.; Kurlan, R.; Gancher, S.T.; Civelli, O.; Grandy, D.; Kidd, K.K. *Arch. Gen. Psychiatry*, **1990**, *47*, 1073-7.
[185] Kennedy, J.L.; Sidenberg, D.G.; Van Tol, H.H.; Kidd, K.K. *Nucleic Acids Res.*, **1991**, *19*, 5801.
[186] Heidbreder, C.A.; Gardner, E.L.; Xi, Z.; Thanos, P.K.; Mugniaini, M.; Hagan, J.J.; Ashby, C.R. Jr. *Brain Res. Rev.*, **2005**, In Press.
[187] Schoemaker, H.; Claustre, Y.; Fage, D.; Rouquier, L.; Chergui, K.; Curet, O.; Oblin, A.; Gonon, F.; Carter, C.; Benavides, J.; Scatton, B. *J. Pharmacol. Exp. Ther.*, **1997**, *280*, 83-97.
[188] Svartengren, J.; Pettersson, E.; Bjork, A. *Biol. Psychiatry*, **1997**, *42*, 247-59.
[189] Oka, M.; Noda, Y.; Ochi, Y.; Furukawa, K.; Une, T.; Kurumiya, S.; Hino, K.; Karasawa, T. *J. Pharmacol. Exp. Ther.*, **1993**, *264*, 158-65.
[190] Schotte, A.; Janssen, P.F.; Gommeren, W.; Luyten, W.H.; Van Gompel, P.; Lesage, A.S.; De Loore, K.; Leysen, J.E. *Psychopharmacology (Berl).* **1996**, *124*, 57-73.
[191] Bymaster, F.P.; Calligaro, D.O.; Falcone, J.F.; Marsh, R.D.; Moore, N.A.; Tye, N.C.; Seeman, P.; Wong, D.T. *Neuropsychopharmacology*, **1996**, *14*, 87-96.
[192] Schmidt, A.W.; Lebel, L.A.; Howard, H.R. Jr.; Zorn, S.H. *Eur. J. Pharmacol.*, **2001**, *425*, 197-201.
[193] Moreland, R.B.; Terranova, M.A.; Chang, R.; Uchic, M.E.; Matulenko, M.A.; Surber, B.W.; Stewart, A.O.; Brioni, J.D. *Eur. J. Pharmacol.*, **2004**, *497*, 147-54.
[194] Kuoppamaki, M.; Syvalahti, E.; Hietala, J. *Eur. J. Pharmacol.*, **1993**, *245*, 179-82.
[195] Neve, K.A.; Neve, R.L., In *The Dopamine Receptors*, Neve, K.A. and Neve, R.L., Ed.; Humana Press: Totowa, New Jersey, **1997**; pp. 27-76.
[196] Millan, M.J.; Gobert, A.; Newman-Tancredi, A.; Lejeune, F.; Cussac, D.; Rivet, J.M.; Audinot, V.; Dubuffet, T.; Lavielle, G. *J. Pharmacol. Exp. Ther.*, **2000**, *293*, 1048-62.
[197] Newman-Tancredi, A.; Assie, M.B.; Leduc, N.; Ormiere, A.M.; Danty, N.; Cosi, C. *Int. J. Neuropsychopharm.*, **2005**, *8*, 1-16.
[198] Szewczak, M.R.; Corbett, R.; Rush, D.K.; Wilmot, C.A.; Conway, P.G.; Strupczewski, J.T.; Cornfeldt, M. *J. Pharmacol. Exp. Ther.*, **1995**, *274*, 1404-13.
[199] Kalkman, H.O.; Subramanian, N.; Hoyer, D. *Neuropsychopharmacology*, **2001**, *25*, 904-14.
[200] Liegeois, J.F.; Bruhwyler, J.; Damas, J.; Rogister, F.; Masereel, B.; Geczy, J.; Delarge, J. *Eur. J. Pharmacol.*, **1995**, *273*, R1-3.
[201] Kassack, M.U.; Hofgen, B.; Decker, M.; Eckstein, N.; Lehmann, J. *Naunyn. Schmiedebergs Arch. Pharmakol.*, **2002**, *366*, 543-50.
[202] Seeman, P.; Corbett, R.; Van Tol, H.H. *Neuropsychopharmacology*, **1997**, *16*, 93-110; discussion 1-35.
[203] Yuan, J.; Chen, X.; Brodbeck, R.; Primus, R.; Braun, J.; Wasley, J.W.; Thurkauf, A. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 2715-8.
[204] Tallman, J.F.; Primus, R.J.; Brodbeck, R.; Cornfield, L.; Meade, R.; Woodruff, K.; Ross, P.; Thurkauf, A.; Gallager, D.W. *J. Pharmacol. Exp. Ther.*, **1997**, *282*, 1011-9.
[205] Pugsley, T.A.; Shih, Y.H.; Whetzel, S.Z.; Zoski, K.; Van Leeuwen, D.; Akunne, H.; Mackenzie, R.; Heffner, T.G.; Wustrow, D.; Wise, L.D. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **2002**, *26*, 219-26.
[206] Millan, M.J.; Gobert, A.; Newman-Tancredi, A.; Lejeune, F.; Cussac, D.; Rivet, J.M.; Audinot, V.; Adhumeau, A.; Brocco, M.; Nicolas, J.P.; Boutin, J.A.; Despaux, N.; Peglion, J.L. *J. Pharmacol. Exp. Ther.*, **2000**, *292*, 38-53.
[207] O'Boyle, K.M.; Waddington, J.L. *J. Neurochem.*, **1987**, *48*, 1039-42.
[208] Glennon, J.; McCreary, A.C.; Ronken, E.; Siarey, R.; Hesselink, M.B.; Feenstra, R.; Van Vliet, B.; Long, S.K.; Kruse, C.G. *Eur. Neuropsychopharmacol.*, **2002**, *12*, 277.
[209] Claustre, Y.; Peretti, D.D.; Brun, P.; Gueudet, C.; Allouard, N.; Alonso, R.; Lourdelet, J.; Oblin, A.; Damoiseau, G.; Francon, D.; Suaud-Chagny, M.F.; Steinberg, R.; Sevrin, M.; Schoemaker, H.;

George, P.; Soubrie, P.; Scatton, B. *Neuropsychopharmacology*, **2003**, *28*, 2064-76.
[210] Scatton, B.; Cohen, C.; Perrault, G.; Oblin, A.; Claustre, Y.; Schoemaker, H.; Sanger, D.J.; Rouquier, L.; Porsolt, R. *Eur. Psychiatry*, **2001**, *16* Suppl 1, 29s-34s.
[211] Needham, P.L.; Atkinson, J.; Skill, M.J.; Heal, D.J. *Psychopharmacol. Bull.*, **1996**, *32*, 123-8.
[212] Arnt, J.; Skarsfeldt, T. *Neuropsychopharmacology*, **1998**, *18*, 63-101.
[213] DeNinno, M.P.; Schoenleber, R.; MacKenzie, R.; Britton, D.R.; Asin, K.E.; Briggs, C.; Trugman, J.M.; Ackerman, M.; Artman, L.; Bednarz, L.; et al. *Eur. J. Pharmacol.*, **1991**, *199*, 209-19.
[214] Kebabian, J.W.; Britton, D.R.; DeNinno, M.P.; Perner, R.; Smith, L.; Jenner, P.; Schoenleber, R.; Williams, M. *Eur. J. Pharmacol.*, **1992**, *229*, 203-9.
[215] Brioni, J.D.; Moreland, R.B.; Cowart, M.; Hsieh, G.C.; Stewart, A.O.; Hedlund, P.; Donnelly-Roberts, D.L.; Nakane, M.; Lynch, J.J., 3rd; Kolasa, T.; Polakowski, J.S.; Osinski, M.A.; Marsh, K.; Andersson, K.E.; Sullivan, J.P. *Proc. Natl. Acad. Sci. U. S. A.*, **2004**, *101*, 6758-63.
[216] Millan, M.J.; Maiofiss, L.; Cussac, D.; Audinot, V.; Boutin, J.A.; Newman-Tancredi, A. *J. Pharmacol. Exp. Ther.*, **2002**, *303*, 791-804.
[217] Arnt, J.; Bogeso, K.P.; Hyttel, J.; Meier, E. *Pharmacol. Toxicol.*, **1988**, *62*, 121-30.
[218] McCreary, A.C.; Ronken, E.; van der Heyden, J.; Herremans, A.; Tuinstra, T.; Long, S.K.; van Scharrenburg, G.J.M., In *Parkinson's disease*, Ronken, E. and van Scharrenburg, G.J.M., Ed.; IOS Press, **2000**
[219] Doll, M.K.; Nichols, D.E.; Kilts, J.D.; Prioleau, C.; Lawler, C.P.; Lewis, M.M.; Mailman, R.B. *J. Med. Chem.*, **1999**, *42*, 935-40.
[220] Shapiro, D.A.; Renock, S.; Arrington, E.; Chiodo, L.A.; Liu, L.X.; Sibley, D.R.; Roth, B.L.; Mailman, R. *Neuropsychopharmacology*, **2003**, *28*, 1400-11.
[221] Bartoszyk, G.D.; Van Amsterdam, C.; Greiner, H.E.; Rautenberg, W.; Russ, H.; Seyfried, C.A. *J. Neural Transm.*, **2004**, *111*, 113-26.
[222] Bristow, L.J.; Kramer, M.S.; Kulagowski, J.; Patel, S.; Ragan, C.I.; Seabrook, G.R. *Trends Pharmacol. Sci.*, **1997**, *18*, 186-8.
[223] Gerlach, M.; Double, K.; Reichmann, H.; Riederer, P. *J. Neural Transm. Suppl.*, **2003**, 167-83.
[224] Olanow, C.W. *Annu. Rev. Med.*, **2004**, *55*, 41-60.
[225] Goldman-Rakic, P.S.; Castner, S.A.; Svensson, T.H.; Siever, L.J.; Williams, G.V. *Psychopharmacology (Berl).* **2004**, *174*, 3-16.
[226] Jardemark, K.; Wadenberg, M.L.; Grillner, P.; Svensson, T.H. *Curr. Opin. Investig. Drugs*, **2002**, *3*, 101-5.
[227] Reynolds, G.P. *J. Psychopharmacol. (Oxf).* **2004**, *18*, 340-5.
[228] Happe, S.; Trenkwalder, C. *CNS Drugs*, **2004**, *18*, 27-36.
[229] Sitaru, C.; Cristea, V.; Florea, S.M. *Rom. J. Intern. Med.*, **1999**, *37*, 275-86.
[230] Zucconi, M.; Ferini-Strambi, L. *Sleep Med.*, **2004**, *5*, 293-9.
[231] Earley, C.J.; Hyland, K.; Allen, R.P. *Mov. Disord.*, **2001**, *16*, 144-9.
[232] Michaud, M.; Soucy, J.P.; Chabli, A.; Lavigne, G.; Montplaisir, J. *J. Neurol.*, **2002**, *249*, 164-70.
[233] Turjanski, N.; Lees, A.J.; Brooks, D.J. *Neurology*, **1999**, *52*, 932-7.
[234] Tribl, G.G.; Asenbaum, S.; Happe, S.; Bonelli, R.M.; Zeitlhofer, J.; Auff, E. *Nucl. Med. Commun.*, **2004**, *25*, 55-60.
[235] Garcia-Borreguero, D.; Larrosa, O.; Granizo, J.J.; de la Llave, Y.; Hening, W.A. *Sleep*, **2004**, *27*, 669-73.
[236] Michaud, M.; Dumont, M.; Selmaoui, B.; Paquet, J.; Fantini, M.L.; Montplaisir, J. *Ann. Neurol.*, **2004**, *55*, 372-80.
[237] Allen, R.; Becker, P.M.; Bogan, R.; Schmidt, M.; Kushida, C.A.; Fry, J.M.; Poceta, J.S.; Winslow, D. *Sleep*, **2004**, *27*, 907-14.
[238] Ahmed, I. *Mo. Med.*, **2002**, *99*, 500-1.
[239] Hening, W.A.; Allen, R.P.; Earley, C.J.; Picchietti, D.L.; Silber, M.H. *Sleep*, **2004**, *27*, 560-83.
[240] Evidente, V.G. *Mov. Disord.*, **2001**, *16*, 579-81.
[241] Stiasny-Kolster, K.; Magerl, W.; Oertel, W.H.; Moller, J.C.; Treede, R.D. *Brain*, **2004**, *127*, 773-82.
[242] Stiasny-Kolster, K.; Oertel, W.H. *Neuropsychobiology*, **2004**, *50*, 65-70.
[243] Baier, P.C.; Trenkwalder, C., In *Animal models of movement disorders*, Ledoux, M., Ed.; Academic Press, **2005**; pp. 755-88.
[244] Clemens, S.; Hochman, S. *J. Neurosci.*, **2004**, *24*, 11337-45.
[245] Clemens, S.; Sawchuk, M.A.; Hochman, S. *Neuroscience*, **2005**.
[246] Weinberger, D.R.; Lipska, B.K. *Schizophr. Res.*, **1995**, *16*, 87-110.
[247] Rollema, H.; Lu, Y.; Schmidt, A.W.; Zorn, S.H. *Eur. J. Pharmacol.*, **1997**, *338*, R3-5.
[248] Ichikawa, J.; Meltzer, H.Y. *J. Pharmacol. Exp. Ther.*, **1999**, *291*, 1227-32.
[249] Goldman-Rakic, P.S. *Adv. Pharmacol.*, **1998**, *42*, 707-11.
[250] Winstanley, C.A.; Chudasama, Y.; Dalley, J.W.; Theobald, D.E.; Glennon, J.C.; Robbins, T.W. *Psychopharmacology (Berl).* **2003**, *167*, 304-14.
[251] Sumiyoshi, T.; Matsui, M.; Nohara, S.; Yamashita, I.; Kurachi, M.; Sumiyoshi, C.; Jayathilake, K.; Meltzer, H.Y. *Am. J. Psychiatry*, **2001**, *158*, 1722-5.
[252] Sumiyoshi, T.; Matsui, M.; Yamashita, I.; Nohara, S.; Kurachi, M.; Uehara, T.; Sumiyoshi, S.; Sumiyoshi, C.; Meltzer, H.Y. *Biol. Psychiatry*, **2001**, *49*, 861-8.
[253] Vialli, M.; Erpsamer, V. *Z. Zellforsch Mikrosk. Anat.*, **1933**, *19*, 743.
[254] Rapport, M.M.; Green, A.A.; Page, I.H. *Fed. Proc.*, **1947**, *6*, 184.
[255] Twarog, B.M.; Page, I.H. *American Journal of Physiology -- Legacy Content*, **1953**, *175*, 157-61.
[256] Gaddum, J.H.; Picarelli, Z.P. *Br. J. Pharmacol.*, **1957**, *12*, 323-8.
[257] Barnes, N.M.; Sharp, T. *Neuropharmacology*, **1999**, *38*, 1083-152.
[258] Dahlstroem, A.; Fuxe, K. *Acta Physiol. Scand.*, **1964**, *62*, SUPPL 232:1-55.
[259] Azmitia, E.C.; Segal, M. *J. Comp. Neurol.*, **1978**, *179*, 641-67.
[260] Conrad, L.C.; Leonard, C.M.; Pfaff, D.W. *J. Comp. Neurol.*, **1974**, *156*, 179-205.
[261] Bobillier, P.; Pettijean, F.; Salvert, D.; Ligier, M.; Seguin, S. *Brain Res.*, **1975**, *85*, 205-10.
[262] Moore, R.Y.; Halaris, A.E.; Jones, B.E. *J. Comp. Neurol.*, **1978**, *180*, 417-38.
[263] Bobillier, P.; Seguin, S.; Petitjean, F.; Salvert, D.; Touret, M.; Jouvet, M. *Brain Res.*, **1976**, *113*, 449-86.
[264] Dray, A.; Gonye, T.J.; Oakley, N.R.; Tanner, T. *Brain Res.*, **1976**, *113*, 45-57.
[265] Fibiger, H.; Miller, J. *Neuroscience*, **1977**, *2*, 975-8.
[266] Giambalvo, C.T.; Snodgrass, S.R. *Brain Res.*, **1978**, *152*, 555-66.
[267] Imai, H.; Steindler, D.A.; Kitai, S.T. *J. Comp. Neurol.*, **1986**, *243*, 363-80.
[268] Parent, A.; Descarries, L.; Beaudet, A. *Neuroscience*, **1981**, *6*, 115-38.
[269] Mamounas, L.A.; Wilson, M.A.; Axt, K.J.; E., M.M., In *Serotonin, CNS receptors and brain function*, Bradley, P.B., Handley, S.L., Cooper, S.L., Key, B.J., Barnes, N.M., and Coote, J.H., Ed.; Pergamon Press, **1992**; Vol *85*, pp. 97-118.
[270] Iyer, R.N.; Bradberry, C.W. *J. Pharmacol. Exp. Ther.*, **1996**, *277*, 40-7.
[271] Shen, H.W.; Hagino, Y.; Kobayashi, H.; Shinohara-Tanaka, K.; Ikeda, K.; Yamamoto, H.; Yamamoto, T.; Lesch, K.P.; Murphy, D.L.; Hall, F.S.; Uhl, G.R.; Sora, I. *Neuropsychopharmacology*, **2004**, *29*, 1790-9.
[272] Bymaster, F.P.; Zhang, W.; Carter, P.A.; Shaw, J.; Chernet, E.; Phebus, L.; Wong, D.T.; Perry, K.W. *Psychopharmacology (Berl).* **2002**, *160*, 353-61.
[273] Tanda, G.; Frau, R.; Di Chiara, G. *Psychopharmacology (Berl).* **1996**, *127*, 83-7.
[274] Miura, H.; Qiao, H.; Kitagami, T.; Ohta, T.; Ozaki, N. *Int. J. Neurosci.*, **2005**, *115*, 367-78.
[275] Jordan, S.; Kramer, G.L.; Zukas, P.K.; Moeller, M.; Petty, F. *Synapse*, **1994**, *18*, 294-7.
[276] Nakayama, K. *Naunyn. Schmiedebergs Arch. Pharmacol.*, **2002**, *365*, 102-5.
[277] Pozzi, L.; Invernizzi, R.; Garavaglia, C.; Samanin, R. *J. Neurochem.*, **1999**, *73*, 1051-7.
[278] Gudelsky, G.A.; Nash, J.F. *J. Neurochem.*, **1996**, *66*, 243-9.
[279] Carlson, J.N.; Visker, K.E.; Nielsen, D.M.; Keller, R.W. Jr.; Glick, S.D. *Brain Res.*, **1996**, *707*, 122-6.
[280] Liegeois, J.F.; Ichikawa, J.; Meltzer, H.Y. *Brain Res.*, **2002**, *947*, 157-65.
[281] Ago, Y.; Koyama, Y.; Baba, A.; Matsuda, T. *Neuropharmacology*, **2003**, *45*, 1050-6.
[282] Denys, D.; Klompmakers, A.A.; Westenberg, H.G. *Psychopharmacology (Berl).* **2004**, *176*, 195-203.
[283] Werkman, T.R.; Olijslagers, J.E.; Perlstein, B.; Jansen, A.H.; McCreary, A.C.; Kruse, C.G.; Wadman, W.J. *Eur. J. Pharmacol.*, **2004**, *506*, 47-53.

[284] Gefvert, O.; Lundberg, T.; Wieselgren, I.M.; Bergstrom, M.; Langstrom, B.; Wiesel, F.; Lindstrom, L. *Eur. Neuropsychopharmacol.*, **2001**, *11*, 105-10.
[285] Gefvert, O.; Bergstrom, M.; Langstrom, B.; Lundberg, T.; Lindstrom, L.; Yates, R. *Psychopharmacology (Berl).* **1998**, *135*, 119-26.
[286] Jones, S.; Kapur, S.; Ziprsky, R.B.; Remmington, G.; Shammi, C.M.; Houle, C.M. *Schizophr. Res.*, **1999**, *36*, 242.
[287] Kuroki, T.; Meltzer, H.Y.; Ichikawa, J. *J. Pharmacol. Exp. Ther.*, **1999**, *288*, 774-81.
[288] Ichikawa, J.; Ishii, H.; Bonaccorso, S.; Fowler, W.L.; O'Laughlin, I.A.; Meltzer, H.Y. *J. Neurochem.*, **2001**, *76*, 1521-31.
[289] Li, Z.; Ichikawa, J.; Dai, J.; Meltzer, H.Y. *Eur. J. Pharmacol.*, **2004**, 493, 75-83.
[290] Moghaddam, B.; Bunney, B.S. *J. Neurochem.*, **1990**, *54*, 1755-60.
[291] Shirazi-Southall, S.; Rodriguez, D.E.; Nomikos, G.G. *Neuropsychopharmacology*, **2002**, *26*, 583-94.
[292] Rollema, H.; Lu, Y.; Schmidt, A.W.; Sprouse, J.S.; Zorn, S.H. *Biol. Psychiatry*, **2000**, *48*, 229-37.
[293] Rasmusson, A.M.; Goldstein, L.E.; Deutch, A.Y.; Bunney, B.S.; Roth, R.H. *Synapse*, **1994**, *18*, 218-24.
[294] Kuroki, T.; Dai, J.; Meltzer, H.Y.; Ichikawa, J. *Brain Res.*, **2000**, *872*, 204-7.
[295] Kuroki, T.; Ichikawa, J.; Dai, J.; Meltzer, H.Y. *Brain Res.*, **1996**, *743*, 357-61.
[296] Wedzony, K.; Mackowiak, M.; Fijal, K.; Golembiowska, K. *Eur. J. Pharmacol.*, **1996**, *305*, 73-8.
[297] Liu, Y.P.; Wilkinson, L.S.; Robbins, T.W. *Psychopharmacology (Berl).* **2004**, *173*, 175-85.
[298] Nakayama, K.; Sakurai, T.; Katsu, H. *Brain Res. Bull.*, **2004**, *63*, 237-41.
[299] Invernizzi, R.W.; Sacchetti, G.; Parini, S.; Acconcia, S.; Samanin, R. *Br. J. Pharmacol.*, **2003**, *139*, 1281-8.
[300] Jordan, S.; Koprivica, V.; Dunn, R.; Tottori, K.; Kikuchi, T.; Altar, C.A. *Eur. J. Pharmacol.*, **2004**, *483*, 45-53.
[301] Ichikawa, J.; Li, Z.; Dai, J.; Meltzer, H.Y. *Brain Res.*, **2002**, *956*, 349-57.
[302] Ichikawa, J.; Dai, J.; Meltzer, H.Y. *Brain Res.*, **2001**, *907*, 151-5.
[303] Yan, Q.S.; Zheng, S.Z.; Yan, S.E. *Brain Res.*, **2004**, *1021*, 82-91.
[304] Bonaccorso, S.; Meltzer, H.Y.; Li, Z.; Dai, J.; Alboszta, A.R.; Ichikawa, J. *Neuropsychopharmacology*, **2002**, *27*, 430-41.
[305] Pehek, E.A.; McFarlane, H.G.; Maguschak, K.; Price, B.; Pluto, C.P. *Brain Res.*, **2001**, *888*, 51-9.
[306] Kuroki, T.; Meltzer, H.Y.; Ichikawa, J. *Brain Res.*, **2003**, *972*, 216-21.
[307] Meltzer, H.Y.; Li, Z.; Kaneda, Y.; Ichikawa, J. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **2003**, *27*, 1159-72.
[308] Pozzi, L.; Acconcia, S.; Ceglia, I.; Invernizzi, R.W.; Samanin, R. *J. Neurochem.*, **2002**, *82*, 93-100.
[309] Alex, K.D.; Yavanian, G.J.; McFarlane, H.G.; Pluto, C.P.; Pehek, E.A. *Synapse*, **2005**, *55*, 242-51.
[310] Godbout, R.; Mantz, J.; Glowinski, J.; Thierry, A.M. *Eur. J. Pharmacol.*, **1991**, *204*, 97-100.
[311] Marek, G.J.; Aghajanian, G.K. *Biol. Psychiatry*, **1998**, *44*, 1118-27.
[312] Wang, Y.; Goldman-Rakic, P.S. *Proc. Natl. Acad. Sci. U. S. A.*, **2004**, *101*, 5093-8.
[313] Olijslagers, J.E.; Werkman, T.R.; McCreary, A.C.; Siarey, R.; Kruse, C.G.; Wadman, W.J. *Neuropharmacology*, **2004**, *46*, 504-10.
[314] Gobbi, G.; Janiri, L. *Eur. Neuropsychopharmacol.*, **1999**, *10*, 43-9.
[315] Ashby, C.R. Jr.; Wang, R.Y. *Brain Res.*, **1990**, *506*, 346-8.
[316] Hertel, P.; Nomikos, G.G.; Iurlo, M.; Svensson, T.H. *Psychopharmacology (Berl).* **1996**, *124*, 74-86.
[317] Millan, M.J.; Dekeyne, A.; Gobert, A. *Neuropharmacology*, **1998**, *37*, 953-5.
[318] Reynolds, G.P.; Yao, Z.; Zhang, X.; Sun, J.; Zhang, Z. *Eur. Neuropsychopharmacol.*, **2005**, *15*, 143-51.
[319] Zhang, Z.J.; Zhang, X.B.; Sha, W.W.; Reynolds, G.P. *Mol. Psychiatry*, **2002**, *7*, 670-1.
[320] Reynolds, G.P.; Zhang, Z.J.; Zhang, X.B. *Lancet*, **2002**, *359*, 2086-7.
[321] Kurata, K.; Ashby, C.R. Jr.; Oberlender, R.; Tanii, Y.; Kurachi, M.; Rini, N.J.; Strecker, R.E. *Synapse*, **1996**, *24*, 313-21.
[322] Chen, J.; Paredes, W.; Van Praag, H.M.; Lowinson, J.H.; Gardner, E.L. *Synapse*, **1992**, *10*, 264-6.
[323] Ashby, C.R. Jr.; Edwards, E.; Wang, R.Y. *Synapse*, **1994**, *17*, 173-81.
[324] Ashby, C.R. Jr.; Zhang, J.Y.; Edwards, E.; Wang, R.Y. *Neuroscience*, **1994**, *60*, 453-62.
[325] Tanda, G.; Frau, R.; Di Chiara, G. *Psychopharmacology (Berl).* **1995**, 119, 15-9.
[326] Cicin-Sain, L.; Jenner, P. *J. Neurol. Sci.*, **1993**, *115*, 105-10.
[327] Wang, R.Y.; Ashby, C.R. Jr.; Edwards, E.; Zhang, J.Y. *J. Clin. Psychiatry*, **1994**, 55 Suppl B, 23-6.
[328] Wang, R.Y.; Ashby, C.R. Jr.; Zhang, J.Y. *Behav. Brain Res.*, **1996**, 73, 7-10.
[329] Porras, G.; Di Matteo, V.; De Deurwaerdere, P.; Esposito, E.; Spampinato, U. *Neuropharmacology*, **2002**, *43*, 1099-109.
[330] Steward, L.J.; Ge, J.; Stowe, R.L.; Brown, D.C.; Bruton, R.K.; Stokes, P.R.; Barnes, N.M. *Br. J. Pharmacol.*, **1996**, *117*, 55-62.
[331] De Deurwaerdere, P.; L'Hirondel, M.; Bonhomme, N.; Lucas, G.; Cheramy, A.; Spampinato, U. *J. Neurochem.*, **1997**, *68*, 195-203.
[332] De Deurwaerdere, P.; Cervo, L.; Stinus, L.; Spampinato, U. *Pharmacol. Biochem. Behav.*, **2002**, *71*, 627-33.
[333] Lacroix, L.P.; Dawson, L.A.; Hagan, J.J.; Heidbreder, C.A. *Synapse*, **2004**, *51*, 158-64.
[334] Frantz, K.J.; Hansson, K.J.; Stouffer, D.G.; Parsons, L.H. *Neuropharmacology*, **2002**, *42*, 170-80.
[335] Joyce, J.N.; Goldsmith, S.G.; Gurevich, E.V. *J. Psychiatr. Res.*, **1997**, *31*, 197-217.
[336] Akil, M.; Pierri, J.N.; Whitehead, R.E.; Edgar, C.L.; Mohila, C.; Sampson, A.R.; Lewis, D.A. *Am. J. Psychiatry*, **1999**, *156*, 1580-9.
[337] Gurevich, E.V.; Joyce, J.N. *Biol. Psychiatry*, **1997**, *42*, 529-45.
[338] Okubo, Y.; Suhara, T.; Suzuki, K.; Kobayashi, K.; Inoue, O.; Terasaki, O.; Someya, Y.; Sassa, T.; Sudo, Y.; Matsushima, E.; Iyo, M.; Tateno, Y.; Toru, M. *Life Sci.*, **2000**, *66*, 2455-64.
[339] Wu, W.L.; Burnett, D.A.; Spring, R.; Greenlee, W.J.; Smith, M.; Favreau, L.; Fawzi, A.; Zhang, H.; Lachowicz, J.E. *J. Med. Chem.*, **2005**, *48*, 680-93.

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